BIOLOGY

A TEXTBOOK FOR HIGHER SECONDARY SCHOOLS

BIOLOGY

A TEXTBOOK FOR HIGHER SECONDARY SCHOOLS

(Classes XI—XII)

PART II

(Volume I)

एन सी ई आर टी
NCERT

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Foreword

THE present volume is the continuation of the Class XI biology textbook It deals with the concepts pertaining to the important areas of biology such as Cell Biology, Genetics, Developmental Biology and Applications of Biology to Human Welfare. All these concepts are developed on the basis of the previous knowledge of the students The authors who are experts in these areas of biology have attempted to enrich the students with contemporary knowledge so that they may develop interest in pursuing higher level of education

I wish to express my thanks to the authors and reviewers for undertaking the work and completing it in so short a time.

Since the writing, reviewing and editing had to be completed in a great hurry to meet the deadline of publication, there are bound to be errors appearing in the book. All suggestions pointing out errors and comments towards the improvement of the book are cordially invited.

S. K. MITRA
Director
National Council of
Educational Research and Training

New Delhi
April 1978

Preface

In the contemporary period, biology has made great advances which have influenced all major branches of human knowledge. The solutions of major problems, like those of food, health and shelter, are expected from the pursuit of biological sciences. The student of biology can acquire a perspective of all facets of the subject by a proper understanding of the structural and functional organization of plants, animals and man. While learning the historical developments and modern trends, the student will have to be aware of the application and significance of his biological background in his day-to-day living. It will help him to enter various academic and professional pursuits or to enter life with greater satisfaction as regards his knowledge of living surroundings, processes and phenomena.

The present textbook for Class XII has been developed to meet the above needs. I would like to thank all members of the editorial board, the authors and the reviewers for completing the work within a short period. Since the writing, reviewing and editing had to be completed in a great hurry to meet the deadline of publication, there are bound to be errors appearing in the book. These will be rectified in a subsequent edition.

We welcome comments, criticism and suggestions from the users of the book.

M. R. N Prasad
Chairman
Biology Editorial Board
for Classes XI - XII

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Contents

UNIT 1

CELL BIOLOGY

CHAPTER 1

Introduction and the Cell Theory

THE CELL is a fundamental morphologic and physiologic unit in the structure of living beings, just as the atom in chemical structure. Both a cell and an atom are composed of simpler components which are so integrated as to exhibit special properties not found in any of the components or in their random mixture. Both exhibit variations in their properties based on different arrangements of parts. Both serve as basic building blocks for more complex structures. However, the analogy cannot be stressed too far; cells can reproduce, whereas atoms cannot. The ability to utilize non-living matter to make living matter is probably the most fundamental property of the cell and cells are the simplest self-duplicating units. Cell biology is that field of biology which deals with the study and knowledge of this fundamental unit of life, the cell.

For many years the branch of biology pertaining to the study of the cell was known as *cytology* However, somehow cytology came to be identified as the science of mere description of the structure of the cell as viewed through a microscope. These days the study of the cell and its components is made through the techniques borrowed from many different branches of sciences, e.g, biochemistry, biophysics, physiology, genetics, molecular biology, etc., and, therefore, it is looked at as a dynamic unit It is in this context that, in recent years, the name cytology has changed to *cell biology*.

Background

It is not easy to trace the development of any field of science because of the lack of full information. Besides, it is true that in science by emphasising the contribution of some, we tend to undermine the painstaking observations and studies of many without which the works attributed to few might not have been possible. However, history has its importance only in highlighting some breakthroughs and in tracing some milestones in the study of a particular field of science. Only in this regard may we trace the history of cell biology.

Dutch scientist, Anton Van Leeuwenhoeck, in 1672. Through his primitive microscope, Leeuwenhoeck was able to describe some protozoa, bacteria, spermatozoa, red blood cells, etc, rather quite accurately An English biologist, Robert Hooke, in 1665, using his primitive microscope (Fig. 1 1A),

observed cells in a section of a cork. He coined the term "Cell" (Latin . *cell—hollow space*) to designate hollow porelike structures which he observed under the microscope. These were actually dead cells of the bark tissue of a plant (Fig 1 1B) Very little was added to these observations for nearly 150 years.

This is by no means an exhaustive list. Study of the cell is progressing at a rapid rate. We have now reached a stage in cell studies that we can anticipate with confidence solutions to many unsolved problems, some of which pertain to two important fields of human life — medicine and agriculture.

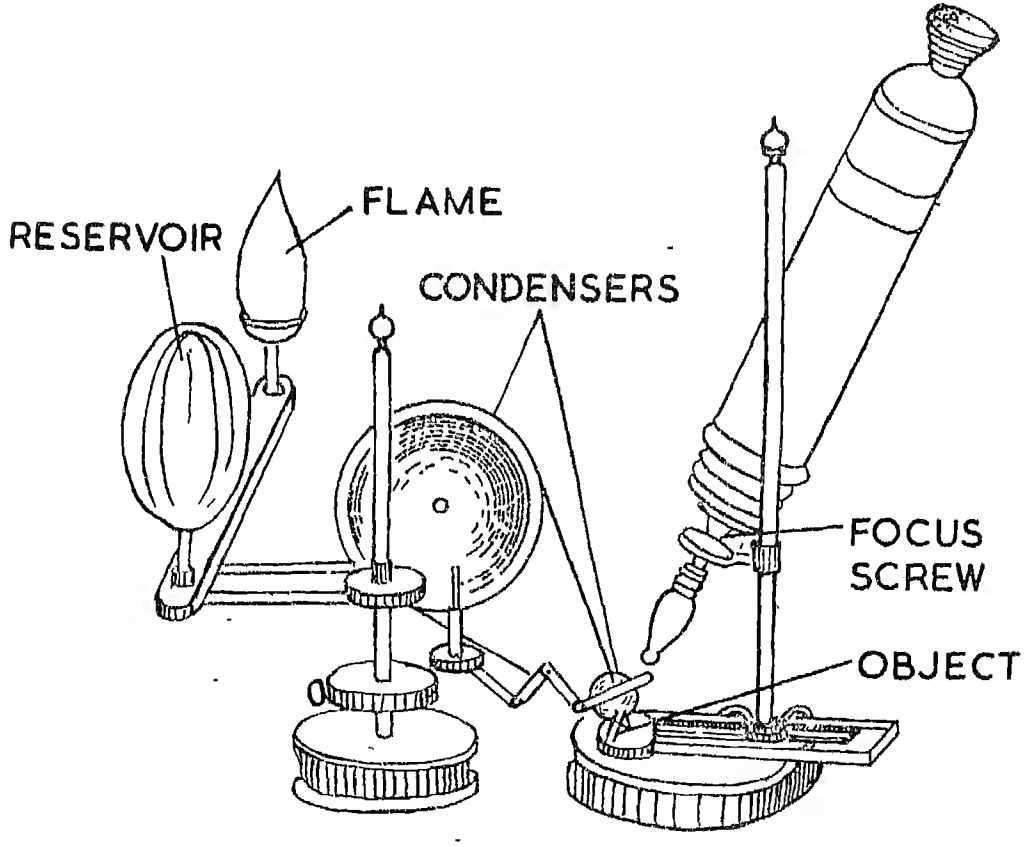


Fig. 1.1A A crude microscope used by Robert Hooke.

However, the nineteenth century witnessed a good deal of studies towards understanding the structure of the cell. History is the catalogue of major advancements in our knowledge. Some of these milestones of progress in cell biology have been listed in appendix 1.

The Cell Theory

We have mentioned earlier that the cell is a morphological and physiological unit of life. This concept is known as the cell theory or cell doctrine. Two German scientists,

M. J. Schleiden and Theodore Schwann in

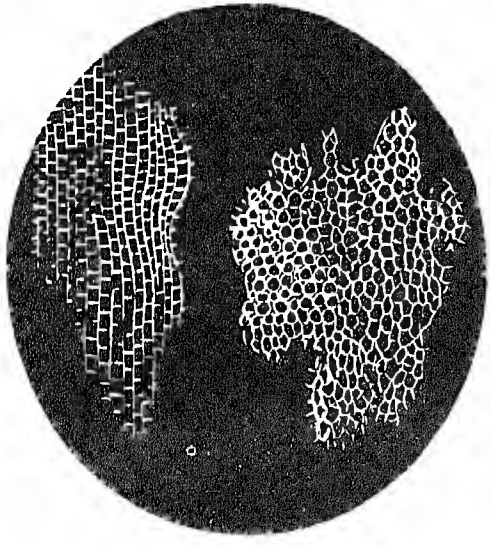


Fig 1.1B Box-like compartments in a cork tissue as observed by Robert Hooke.

their studies in plants and animals in 1838 and 1839, respectively, described why the cell should be considered as a unit of life. Hence, they are credited for having formulated the now familiar cell theory. However, it should be noted that they were not the first to conceive the idea of the cell as a basic unit. The French scientist, H. J Dutrochet, in 1824 boiled some tissues in an acid and separated the cells and thought that the tissues were composed of the smaller units—the cells There were several scientists who contributed to the knowledge of the structure of the cell in the nineteenth century. Schleiden and Schwann put all these developments together and formally spelled out the observations into a convincing doctrine that cells containing nuclei were the structural basis of the organisation of both plants and animals. Another German scientist, Rudolf Virchow, made another important generalization: *cells come only from pre-existing cells.* Putting all these observations together, the cell theory can be stated as follows

1. Cells arise only from pre-existing cells
2. All organisms are composed of cells and cell products.
3. Cells are the structural and functional units of life

The cell theory is one of the important generalizations of biology and ranks with Charles Darwin's *theory of evolution* and the *theory of the gene* of modern biology.

EXERCISES

1. Why should we study cell biology?
2. Can you mention three biological generalizations of great significance?
3. What is the cell theory?
4. Which do you think is the greatest event in the history of cell biology? Why?

CHAPTER 2

Tools and Techniques

BEFORE 1650, nothing was known about the cell and even its very existence could not be suspected because nearly all cells are too small to be seen with the naked eye. It was only after the invention of the microscope that we could see objects which were beyond the perception of the human eye. Instruments like the microscope, therefore, act as extended senses. Light microscopes and electron microscopes have opened us a new microworld ordinarily invisible to us Ordinarily, we can see only a minute portion of the visible light of the electromagnetic spectrum. By utilizing photosensitive surfaces we can detect the long infra-red rays on one side of the spectrum and the short ultra-violet rays, X-rays, etc., on the other. Just like tools, techniques also enable us to know a great deal about things which we may not ever see, like atoms, molecules and other structures. Hence, there is a direct relation of the progress in the development of new and better instruments and techniques to the progress and refinement of our knowledge of science. This is particularly true of our knowledge of cell biology. We will describe a few of the important tools and techniques being used in the modern study of the cell

Microscopy

The human eye cannot see objects smaller than 100 microns. This means that any two points closer than the distance of 100 microns cannot be distinguished as two distinct points by our eyes. They may appear as one or blurred images. This ability to distinguish two close points as two separate points is known as *resolving power*. Hence, the resolving power of the human eye is 100 microns. The microscope is an instrument which magnifies as well as resolves the objects seen through it. However, in order to see the objects we have to use some kind of illumination. The resolution of a microscope also depends on the kind of illumination used. Generally, objects closer than one-half the wave length of the illuminating light cannot be clearly distinguished in a light microscope. The wave lengths of the visible spectrum of light range from 4000 A° to 8000 A°. Taking an average of 6000 A° as the wave length, the resolving power of a light microscope will be about 3000 A° or 0.3 microns. Thus, even a light microscope has its limitations and cannot enable us to see objects smaller than 0.30 to 0.25 microns. Since many parts of cells have smaller

dimensions, their presence and structure were undetected until the invention of the *electron microscope* (Fig. 2.1A).

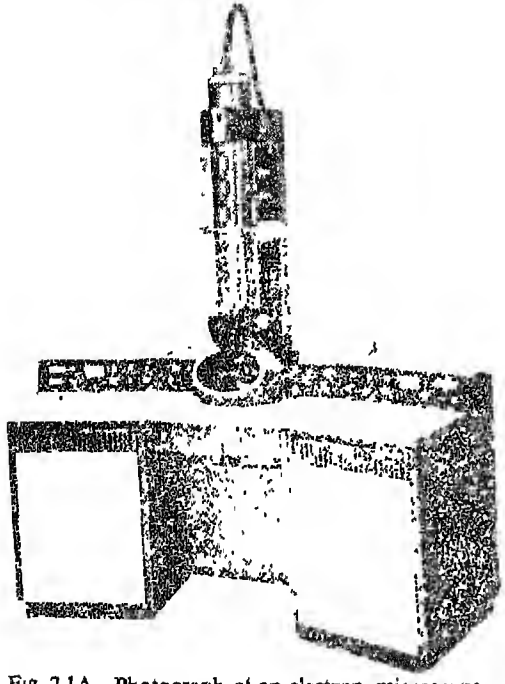


Fig 2.1A Photograph of an electron microscope—EM 300 PHILIPS

The structure of a compound light (optical) microscope (Fig. 2.2) is quite simple. It consists of a system of two lenses: (*i*) an objective lens and (*ii*) an ocular lens and a condenser. The objective lens produces an initial image of the object. The quality of this image determines the resolution of the image. The ocular lens (eye-piece) magnifies the initial aerial image and produces the final image. The function of the condenser is to direct a light beam on to the specimen.

The electron microscope utilizes a stream of high speed electrons instead of light waves. The wave length of electrons is determined by the voltage at which they are generated. At 50,000 volts, they have a wave length of about 0.50 A°. Thus, the resolving power of the electron microscope can be one-half of 0.50 A°; i e, 0.25 A° However, due to technical difficulties, the resolving power below 10 A° is rarely achieved With this resolving power, the electron microscope actually opened up a new domain to the cell biologist, by making a number of cellular structures visible with remarkable details (Fig. 2.1B).

The electron microscope is based on the response of electrons to electromagnetic fields. A metal filament heated in a vacuum emits electrons which follow a straight path similar to light rays. The beam of electrons is focussed with the help of electromagnetic lenses which are actually coils of wire enclosed in a soft iron casing. After passing through the object, the beam is deflected by the electromagnetic lens which acts like an objective lens. The resulting image is passed through another lens, the projector lens, which throws the final magnified image on to a fluorescent screen. A photographic plate may be substituted for the fluorescent screen and exposed. Such photographs are the electron micrographs. Thus, there seems to be some sort of similarity in the make-up of the compound light microscope and the electron microscope (Fig. 2.3). But the latter is a much more sophisticated and expensive instrument.

In order to study cells and tissues under an electron microscope, they have to be killed and fixed in certain chemical solutions, cut and stained with some dyes or stains to provide contrast. Living cells cannot be observed under the electron microscope. Hence, doubts are often raised regarding the reality of structures and details observed through these microscopes.

The *phase contrast* microscope is a device which enables us to observe living cells and tissues, although its resolution is not better than an ordinary microscope since it is also a kind of light microscope and utilizes light

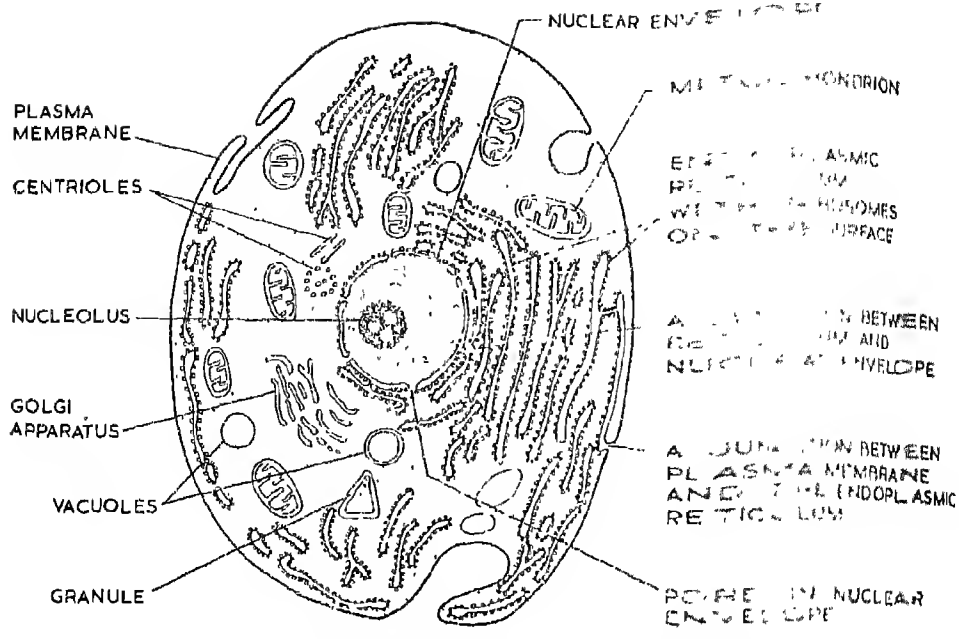


Fig 2.1B Diagrammatic ultrastructure of a typical cell.

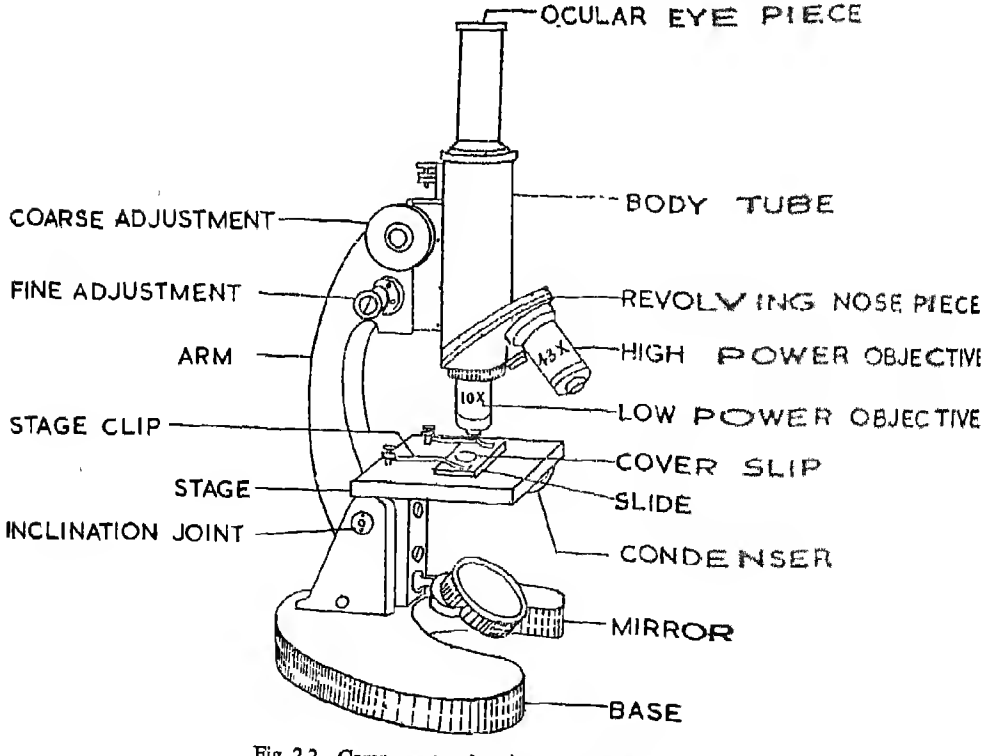


Fig. 2.2 Components of a light microscope.

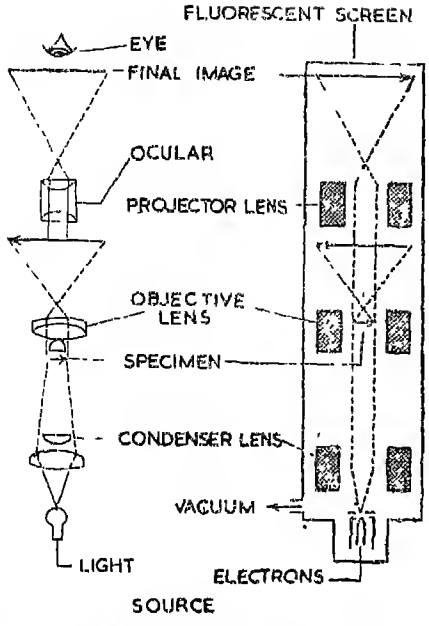


Fig. 2.3 A diagram showing the similarities and differences between a light and an electron microscope

rays for illumination. It is based on the principle that intensity (brightness) is proportional to the square of its amplitude of light waves. When the light rays are parallel, their amplitudes are said to be in the same phase. However, if they are progressing at different angles, they are said to be out of phase.

A phase difference in the light rays passing through the various components of the cell and those reaching directly without passing through the object is created in the phase microscope. Due to these phase differences, intensity variations are produced, resulting in the maximum contrast. Because of the contrast, we can observe the cell and its various components in their living state (Fig. 2.4A).

There are several kinds of light microscopes: for example, interference microscope, fluorescent microscope, polarising microscope, UV microscope, etc., each developed for a specific need. The interference microscope is used for quantitative studies of various macromolecules of the cell components.

When certain chemical substances are irradiated with UV light, they absorb the radiation and emit visible light. Such chemicals are known as *fluorochromes*. Some such fluorochromes bind with the specific parts of molecules of the cell structures. The kind of microscope which is used to localize the cell structures, or to detect minute quantities of materials with the help of fluorochromes is known as *fluorescent microscope* (Fig. 2.4B). The *polarising* microscope can detect regions in cells where constituents are disposed in highly ordered array (Fig. 2.4C). This is done with the help of a prism, the *polarizer* built into the microscope. The *ultra-violet* microscope makes use of the fact that certain substances in the cell, for example nucleic acids (RNA and DNA), strongly absorb ultra-violet light. Pictures taken through the ultra-violet microscope show the locations of high concentrations of nucleic acids as regions darker than the rest of the cell.

Cytochemistry

The technique of cytochemistry is generally used to locate specific constituents within the cells. This is done by producing a colour contrast or special deposits at the specific sites where the constituent is present in the cell. Dyes which can bind a particular substance can be used. For example, *Schiff's reagent* reacts, under certain conditions, with DNA only and can be used to localize the presence of DNA in a cell. Similarly, under proper conditions, substrates of enzymes can be used to localize the distribution of enzymes. This is possible since, under certain conditions, the reactions of some enzymes with substrates can produce insoluble pro-

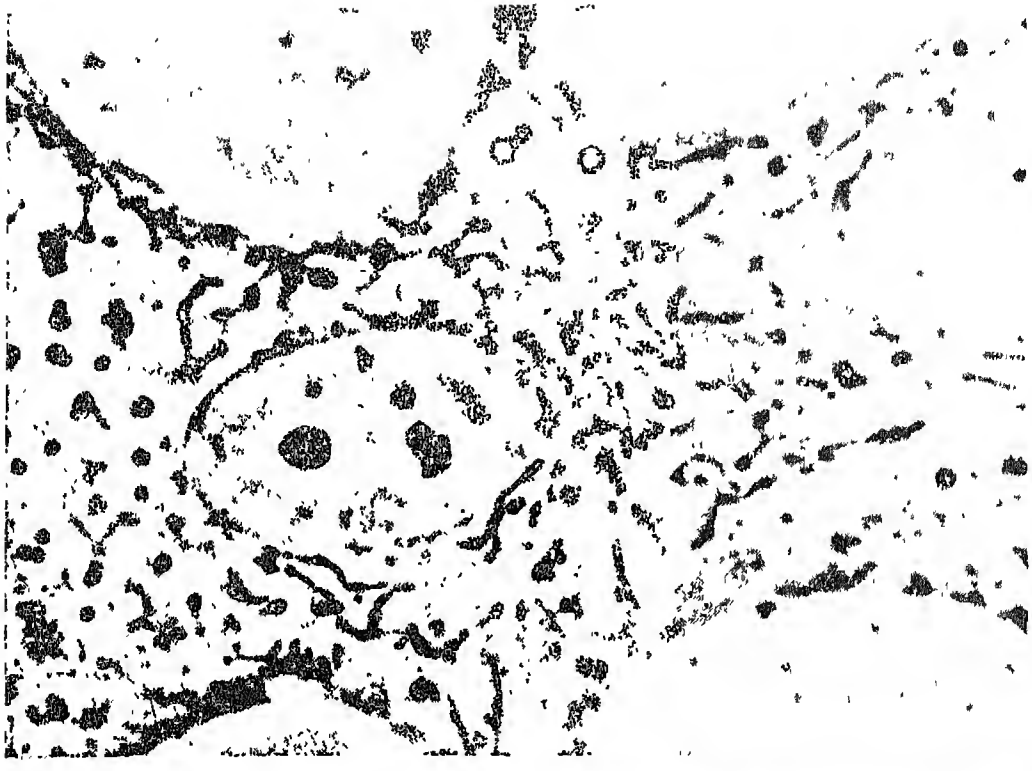


Fig. 2.4A A living cell as observed through a phase-contrast microscope.

ducts, visible through the microscope. Under proper conditions, some of these techniques can even be used for quantitative studies since the amount of dye taken up may be directly proportional to the amount of the stained components. A special technique of *microspectrophotometry* is developed for such quantitative analysis. It is now possible to localize a large number of cell constituents by the methods of cytochemistry. It is even possible to use some of these techniques to visualize the distribution through the electron microscope enabling us to obtain greater precision of locations.

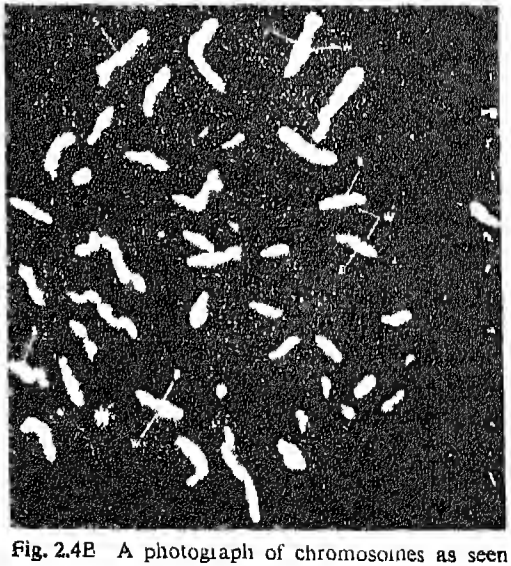


Fig. 2.4B A photograph of chromosomes as seen under a fluorescent microscope.

Autoradiography

One of the important techniques used to study the synthesis of molecules and to trace

metabolic events in cells is called *autoradiography*. Some radioactive precursors which are building blocks in the synthesis of macromolecules are used to trace the metabolic activities of the macromolecules The most widely used isotopes are tritium (^{3}H), carbon (^{14}C) and phosphorus (^{32}P). These isotopes are incorporated into the precursors which are administered into cells and their pathways are followed by fixing the cells at various intervals Tritium or carbon-labelled thymidine is used for studying the synthesis of DNA; tritium or carbon-labelled uridine is used for studying the synthesis of RNA, and tritiated or carbon-labelled amino acids are used for tracing protein synthesis. In this technique, after the administration of radioactive precursors, the cells are fixed and sectioned if necessary These sections are coated with photographic emulsion The sections are exposed in dark for certain length of time and then developed just like ordinary photography. Emission of radiations from the radioactive substance reduces, just like light rays, the silver salt of the emulsion to produce metallic silver grains These silver grains form the image, just like the photographic film The presence, location and amount of silver grains can furnish data which can be useful in interpreting events concerning the precursors and macromolecules in which they are incorporated (Fig. 2.5). Autoradiography is very useful for studying dynamic aspects of the cell constituents For example, if radioactive uridine is fed to cells and these are fixed only a few minutes later, almost all grains will be found on nuclei, suggesting that RNA is synthesised in nuclei and not in cytoplasm. If the interval between the exposure and fixation is prolonged by an

Fig. 2.4C A view through a polarising microscope — a nucleus in mitosis.

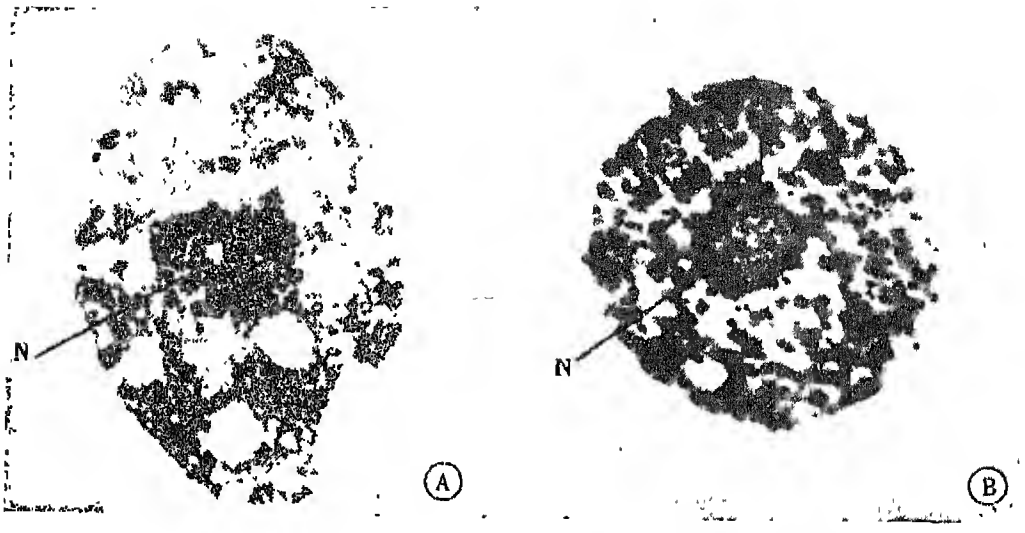


Fig. 2.5 Showing silver grains in (*A*) nucleus and (*B*) cytoplasm.

hour or two, most of the grains are observed in cytoplasm. This means that RNA is synthesised in nucleus and is transported to cytoplasm. Such findings are very valuable in metabolic studies of cells.

Cell Fractionation

Cell fractionation is another important and versatile technique for studying cell chemistry (Fig. 2.6). Tissues and cells are homogenized in certain media which preserve cell structures in good condition. The homogenates of cell fragments are placed in a test-tube and are centrifuged. The sedimentation of these fragments of cell structures depends chiefly on their weight and size. Since most of the cell organelles differ in their sizes and weights, they can be separated easily. This method of separating them by centrifugation is known as *differential centrifugation*. Solutions of sucrose or other sugars are generally used because they provide proper density and prevent clumping of the cell fragments. With this method, one can easily separate almost intact and pure fractions of nuclei, mitochondria, chloroplasts, lysosomes, nucleoli, microsomes (fragments of endoplasmic reticulum), etc. These fractions can then be subjected to biochemical analyses.

Biochemical and Biophysical Techniques

Only a very brief outline of some of the most widely used methods can be given here

(*i*) The pH of a solution may be accurately measured by a meter A potential difference related to (H^+) is set up and, after amplification, may be read off as an actual pH value.

(*ii*) Biochemical compounds have a property of showing maximum absorption of light of different wave lengths. Very small amounts of biochemical substances can be quantitatively determined by measuring the percentage of absorption they show at selected

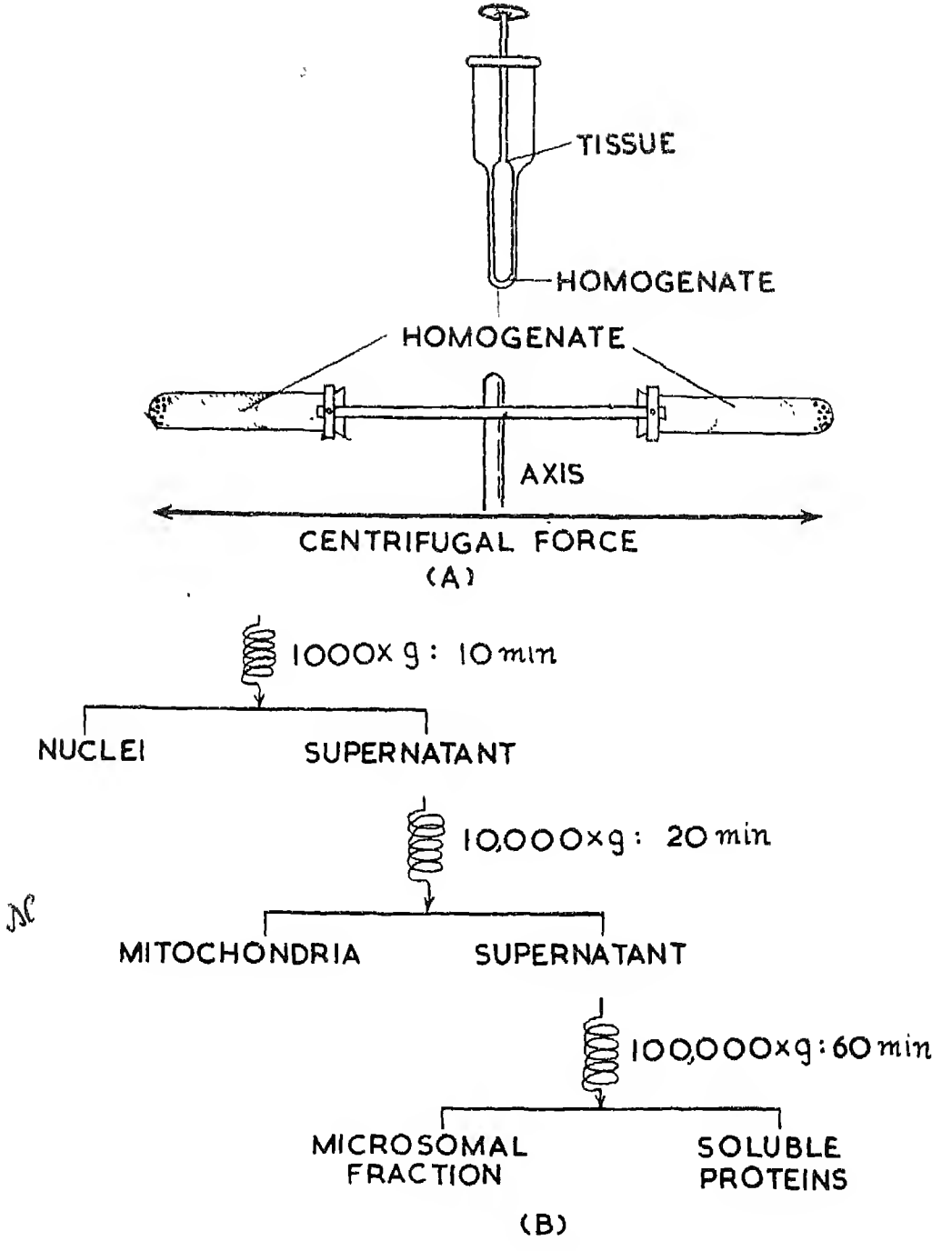


Fig. 2.6 Technique for the isolation of different cell fractions.

wave lengths as compared with solutions of known concentrations Sometimes, colours develop as a result of some reactions Such coloured solutions can be used for absorption studies as they can yield better accuracy. The method is called *spectrophotometry*.

(*iii*) It is possible to separate small quantities of organic (and inorganic) compounds according to the rate they travel along a piece of paper, or a column of suitable material. This allows detection and identification of minute amounts of reaction products as the rate of travel of any given substance under controlled condition is constant and, thus, unknowns can be compared with standard solutions. The technique is called *chromatography*.

(*iv*) Ion exchange resins are used for the extraction and purification of organic compounds of small molecular groups and ionisable groups such as amino acids.

(*v*) *Ultracentrifuges* are centrifuges with very high speeds which make it possible to separate not only cell constituents but even large molecular species of different densities.

(*vi*) A very sophisticated and complex method for analysing the structure of molecules in crystalline form is *X-ray crystallography*. It is based on the measurements of diffraction patterns of X-rays as they pass through a crystal of the substance(Fig. 2.7). Such studies can reveal important information relating to the arrangements of atoms in the molecular structure of substances like enzymes. The technique is also of classical interest as it was used by Wilkins, Watson and Crick to determine the molecular configuration of double helix of DNA.

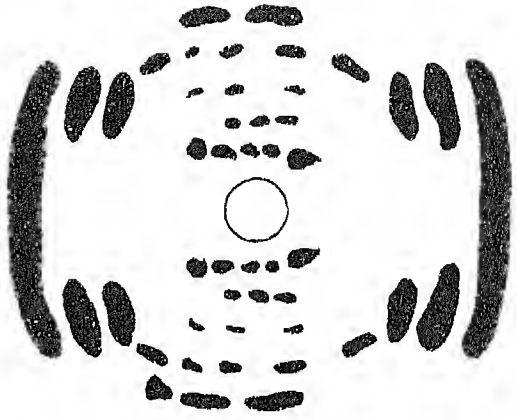


Fig. 2.7 X-ray diffraction pattern of DNA.

Tissue Culture

In modern cell research, tissue culture is an important method. Pieces of tissues or isolated cells can be cultivated in special fluid media in a variety of glass or plastic tubes, vials or bottles. In proper media, cells cannot only remain alive for a long period, but can also grow and multiply. Cells in culture can be used for solving many most important problems of cell metabolism; for example, in cancer research, tissue culture cells are widely used. Leucocyte culture cells from human and animals are used for chromosome preparations in cytogenetic studies. Such studies on man have provided interesting information regarding the correlation of chromosomal abnormalities and congenital diseases. Plant cell cultures have been utilized for studying processes of differentiation and growth. Tissue culture cells have also been used for genetic engineering studies

These are only a few of the most important techniques used in modern studies of cells. It is obvious that the cell biologist has a large varieties of instruments and techniques

available to him to study the secrets of the cell. However, any one tool is inadequate and often one has to use several methods to tackle any particular problem under investigation. Such knowledge as we have gathered of the cell has strengthened our belief that the cell is the basis of life and has, at the same time, made us aware of yet how little we know of the infinite complexities of this tiny little unit of life.

EXERCISES

1. Give a brief account of the biochemical methods used in the study of a cell.
2. Discuss the statement that progress in cell biology is directly linked up to the progress in the development of techniques and tools used in it.
3. Describe the technique of autoradiography.
4. What is tissue culture? How is it useful in the study of cell biology?
5. Compare the electron microscope with the light microscope and discuss their merits and demerits
6. What is the difference between resolution and magnification?
7. Discuss the working and usefulness of the phase-contrast microscope.

CHAPTER 3

Cells—Shape, Size and Composition

ACCORDING to the cell theory, all living things are either single-celled or composed of colonies of cells and that the cell is the real unit of life. Naturally, then, if we can know what a cell is made of and how it works, we can understand what life is. However, this is not an easy job. Although most cells are tiny microscopic in size, they are infinitely complex units. Cell biology has witnessed great strides in the direction of unravelling the secrets of life and a lot is yet to be understood about the most complex system of the cell.

The cell can be defined as a block of protoplasm surrounded by a membrane and often divided into compartments delimited by membranes or differential densities of the components. But this definition is inadequate since the cell is not a mere aggregation of static components. The cell is not even a complex system of heterogeneous molecules. The cell is actually an ever-changing dynamic unit adapting itself to the role it is required to play. The cell is a highly ordered system of mutually interdependent and interacting components. Now, with this realization, we may go ahead to describe the cell. There is nothing like a typical cell. Cells differ in shapes, sizes and contents according to the functions they perform.

Size

Although some cells are visible to the naked eye, most are microscopic in size within limits of 10 to 100 microns (Fig. 3.1A). For example, an ostrich egg (Fig. 3.1B) is the largest animal cell, as big as 170 × 135 mm while the smallest known cell is that of a bacterium of pleuropneumonia, called PPLO (Pleuropneumonialike organisms) which may measure 0.1 to 0.5 micron in size (Fig. 3.1C). Thus, the size of PPLO may not be more than 1000 to 5000 times that of a hydrogen atom.

In plants, certain algae have gigantic cells An alga, *Acetabularia* (Fig. 3.2), consists of a single cell about ten centimetres in length. In the human body, nerve cells are the largest, often about 90 µm in length. Muscle cells are also large but most other cells of the kidney, the liver, the intestine, etc., are between 20 to 30 microns in diameter. In most cases, the size is correlated to the function of the cell. In size, the surface area of the cell membrane is very important. A cell takes nutrients from its environment and gives off waste

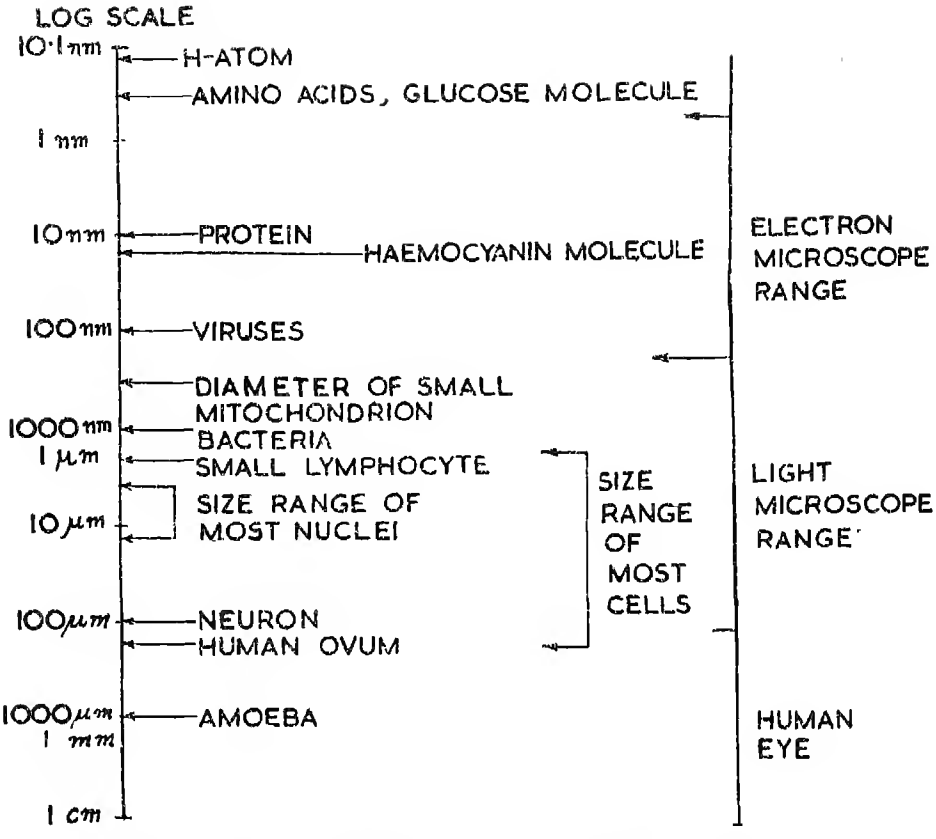


Fig. 3.1A Logarithmic scale to show the size range of cells, molecules and atoms and the range of resolution of various microscopes. (1 nm=10^{-9} m=10^{-6} m m=10^{-3} μm=10Å)

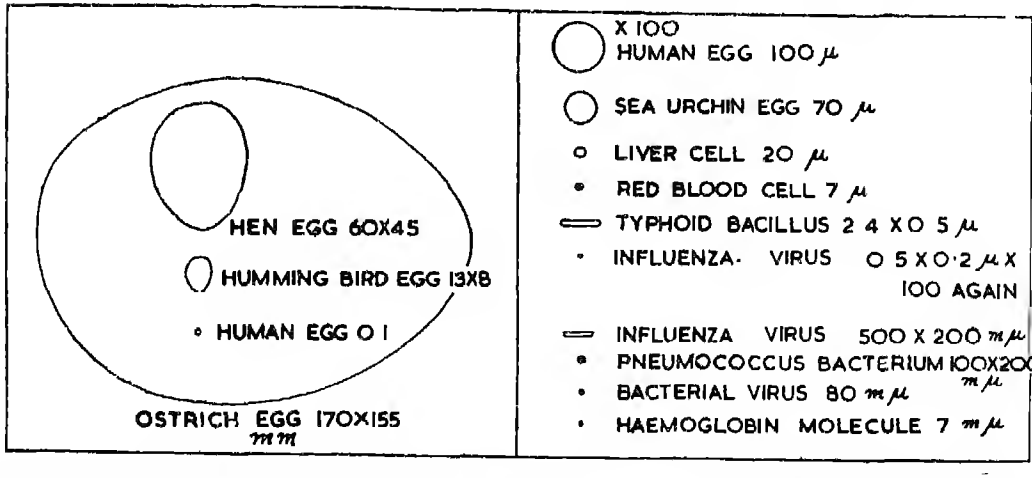


Fig. 3.1B A scale of sizes of different kinds of cells, with the bacterial virus and the haemoglobin molecule included for comparative purposes. The ostrich egg and the avian egg within it are reduced by one-half.

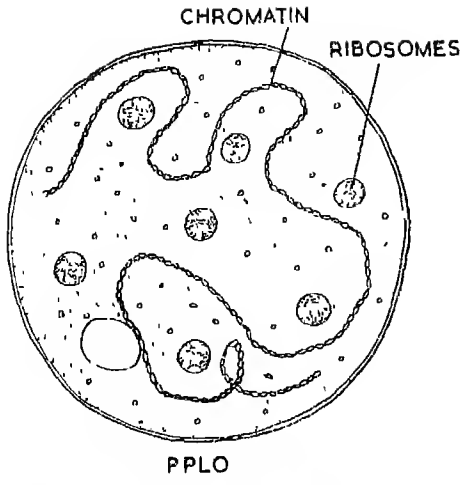


Fig. 3.1C Ultrastructure of pleuropneumonialike organisms (PPLO)

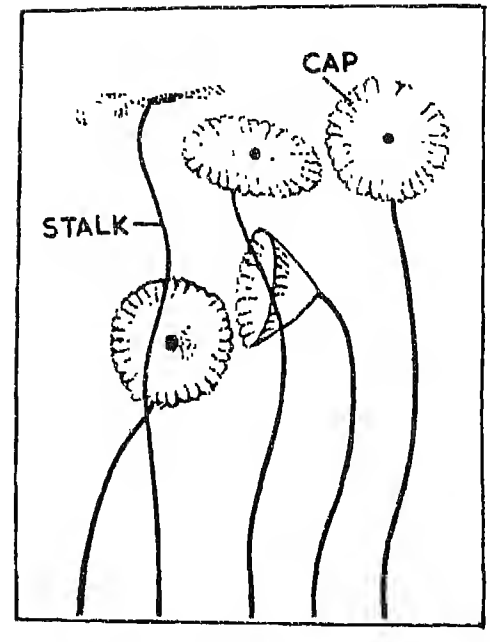


Fig 3 2 Single-celled alga — *Acetabularia.*

through its surface. The total surface area is critical because it affects the cell's ability to exchange material with its environment. Generally, a cell which is metabolically very active cannot have a very large volume. The amount of nutrients needed for metabolism depends on the cell volume. Small bodies have more surface per unit volume than large bodies as can be seen from Fig. 3.3. Hence, no cell can have a volume whose

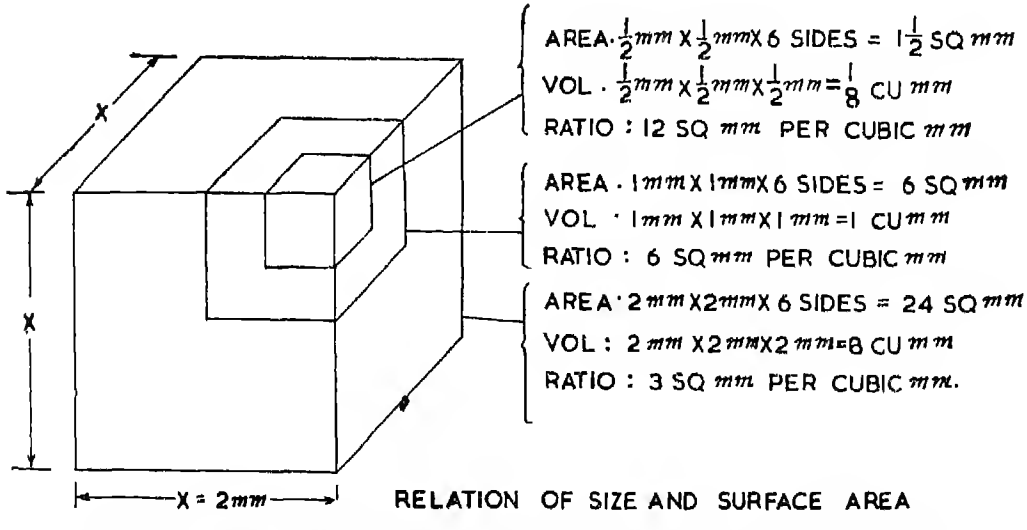


Fig. 3.3 The effect of the size on the surface area. The surface per unit volume enclosed increases as the volume is diminished.

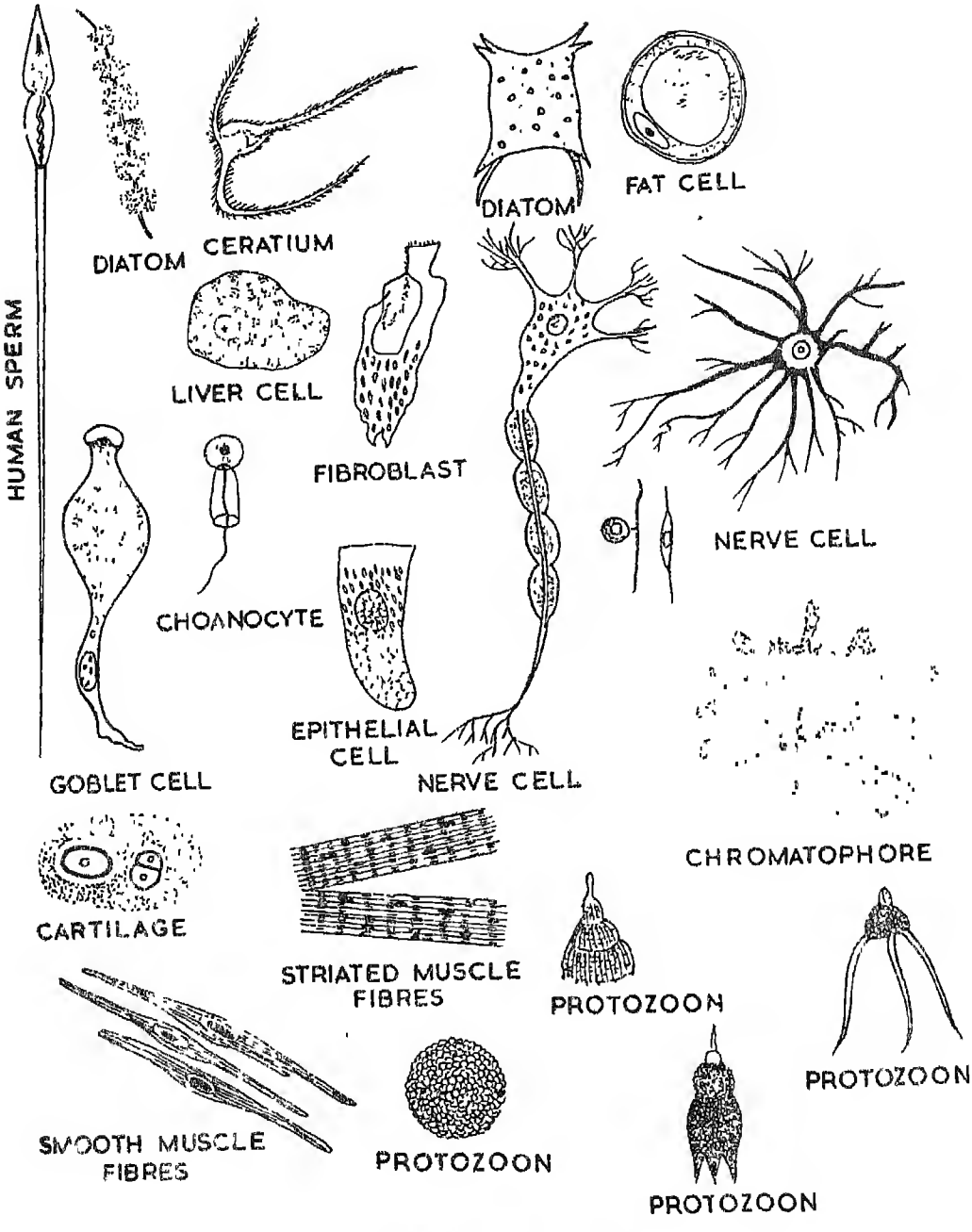


Fig. 3.4 Various kinds of cells.

metabolic requirements exceed the exchange capability of its surface. At least two major factors restrict the cell size : (*i*) the cell's requirement of oxygen and other materials from its environment and (*ii*) the regulating ability of its nucleus. There is no relation between the size of the cells and the body size of an organism, i.e., the elephant or whale does not have large cells.

Shape

As regards shapes, cells show even greater diversity than in sizes (Fig. 3.4). Here, again, cells demonstrate the existence of close relationship between a cell's form and function. Some cells like those of *Amoeba* and white blood cells can change their shape continuously while most seem to maintain their shapes stable all through their existence. Free living cells such as protozoans and algae show a great range of forms from simple spheres to bizarre and complex. Among the cells of multicellular forms, a variety of shapes are present in the same organism. Cell shape is mainly controlled by such factors as function, age, viscosity, cell wall, external pressures or tensions and internal or external skeleton. Nerve and muscle cells are good examples of cells peculiarly adapted to a particular function.

Cell Number

Unicellular organisms are formed of single cells. Multicellular organisms are formed of many cells, which in turn may be of many types. The cortex of the human brain may consist of nine billion two hundred million cells. The human blood has 30 quadrillion (30×10^{15}) cells and the human body weighing about 60 kg may consist of 60×10^{15} cells. However, all multicellular organisms begin with a single cell—zygote. All other cells are derived from multiple divisions during the growth of an organism.

Physical Structure

We must again emphasize that all cells have specialized roles and offer a great diversity of form and function. Hence, the generalized cell that we may describe here cannot be considered as the typical cell. From the varieties, we may, for the sake of simplicity, consider three representative types of cells as generalized cells. The cells of micro-organisms such as bacteria (Fig. 3 5A) are different from the cells of higher organisms like plants and animals. These cells do not possess well-formed nucleus and nuclear membrane which separate the cytoplasm from the nucleus and are, therefore, known as *prokaryotic* cells. These cells, instead of a nucleus, have a nuclear zone called nucleoid. In higher organisms, cells possess definite nuclear membranes forming two distinct compartments of the cytoplasm and the nucleus. These cells are called *eukaryotic* cells. Among the eukaryotic cells, the plant cells possess cellulose cell walls, large vacuoles and plastids and, thus, differ from the animal cells which lack all these (Fig. 3.5B) In spite of these differences, all of them may show some common general features.

A typical cell may be regarded to be composed of two main compartments—the nucleus and the cytoplasm.

All cells possess a cell membrane or plasma membrane which encloses the internal parts and allows some materials to pass in and out but excludes others. Such a membrane is said to be selectively permeable. All plant and animal cells possess a large spherical body called the *nucleus*. The nucleus contains one or more spherical,

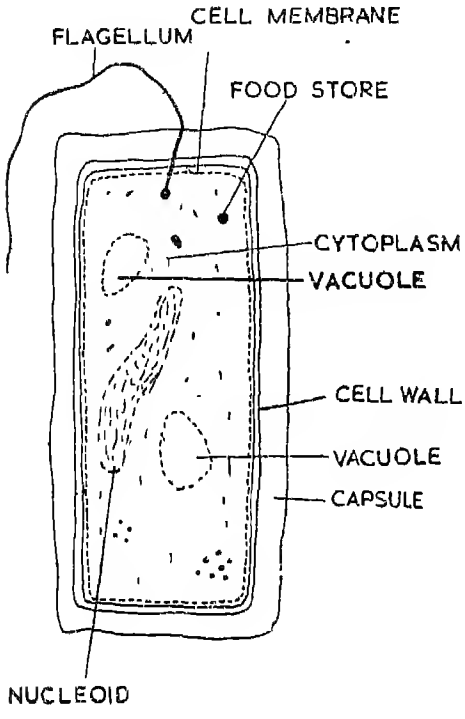


Fig. 3.5A Diagrammatic sketch of a bacterial cell.

dense bodies called *nucleoli* which are rich in ribonucleic acid—RNA. In the nucleus, there are also threadlike bodies called *chromosomes* which can be seen at certain times Chromosomes bear the genes which contain the hereditary material deoxyribonucleic acid—DNA. DNA is ultimately responsible for directing the functions of cells. The nucleus is bounded by a membrane and the cytoplasm lies between the nuclear and plasma membranes.

Throughout the cytoplasm, there are, characteristically, small membrane-bounded bodies which are called *organelles* Briefly, these bodies are threadlike mitochondria, which extract energy from food-stuffs and convert it into biologically useful form; plastids (chloroplasts) in plant cells, which contain pigments for transforming the sun's radiant energy into the chemical energy of molecules such as sugars; the Golgi apparatus, a system of canals commonly seen in secretory cells; and lysosomes which contain enzymes capable of digesting food particles and cellular substances.

Above is the description of cells as viewed under a light microscope. If we observe a cell through an electron microscope, we can see much fine structural organization of the organelles just described as well as some other details of the organization of the cell. For example, endoplasmic reticulum, a membrance network of channels which transports certain materials within the cell, cannot be observed under an ordinary light microscope. With the help of the electron microscope, we can see that on the outer surface of some of these tubular channels lie small spherical ribosomes which are involved in the synthesis of proteins. In the animal cells, a pair of dotlike objects, the centrioles, can be seen lying near the nucleus. They serve an important function in cell division (see Fig. 2.1B).

Chemical Composition

As indicated earlier, cells have a large and heterogeneous population of elements and molecules. Despite this chemical heterogeneity, it is possible to classify chemical constituents of cells. The knowledge of these chemical constituents, their abundance, proportions and locations within the cell is useful for our understanding of its biological organization and function.

By weight, the most abundant elements found in the animal cells are oxygen 65%, carbon 18%, hydrogen 10%, and nitrogen 2.5%, followed by calcium, sodium, ranging in weight from 0.15% to 2%. However, these figures may be somewhat misleading because if the percentage of the relative

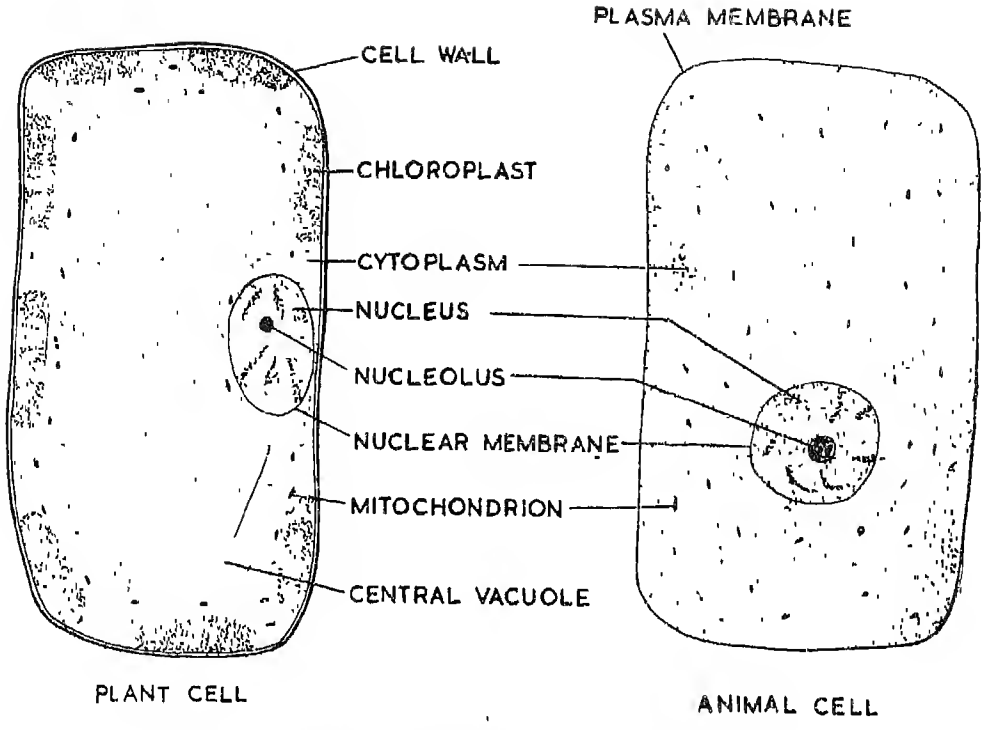


Fig. 3.5B Comparison between (*A*) plant cell and (*B*) animal cell

abundance of elements is considered, hydrogen (60%), oxygen (20%), and carbon (11%) are greatly in excess of all other elements, the next nearest being nitrogen at 2.4%. The predominance of hydrogen and oxygen is accounted by the fact that 60 to 90% (by weight) of protoplasm is water, the water content being the highest in the embryonic cells and decreasing progressively with age. Its amount also differs in relation to metabolism being only 20% in the bone cells and 85% in the brain cells. Hydrogen and oxygen combined with carbon and nitrogen make up the major constituents of cells, namely. proteins (7-20%) carbohydrates (1-2%) and lipid (1-3%). The inorganic elements of cells account for 1-2% by weight and include not only the elements mentioned above but also trace elements such as magnesium, chlorine, iron, manganese and copper which have significant roles to play in enzyme action and other metabolic processes.

The relative abundance of the main chemical classes and the number of molecules per cell relative to DNA are shown in table 3.1.

TABLE 3.1

Percentage and Number of Molecules Per Cell Relative to DNA

Molecule	Per cent	Number of molecules relative to DNA
DNA	0.4	1
Water	8.0	1.2×10^7
Protein	9.0	7.0×10^2
Carbohydrates	2.0	14.0×10^3
Lipid	2.0	7.0×10^3
RNA	0.7	4.4×10^1
Other organic compounds	0.4	4.0×10^3
Inorganic compounds	1.5	6.8×10^4

These figures do not indicate the relative importance of a particular element of molecule from the point of view of the cell structure and function. DNA and RNA occur in very small amounts but are highly significant in heredity and control of protein synthesis. Water is the most abundant molecule and without it cell processes would be impossible Proteins are found associated with membranes and also as enzymes or hormones. Carbohydrates occur as storage products, and are associated with proteins in cell secretions. Lipids occur in membranes and in storage inclusions.

In summary, therefore, the cell can be defined as a systematically organized community of molecular populations in dynamic interaction. It has a morphological, chemical and physical organization which enables it to assimilate, grow and reproduce. The cell is a part of the physical universe and is subjected to the same laws as other physical objects, but it differs from the others as it has the power of self-regulation and adaptability which other objects do not have.

EXERCISES

1. Suppose, a cell is 0.1mm in diameter. What is its size in nanometers ?
2. Is it correct to state that all biological processes have molecular basis ?
3. What is a typical cell ? Compare a plant cell with an animal cell.
4. Why is the cell considered as the basic unit of life ?

CHAPTER 4

Cell Wall and Plasma Membrane

Cell Wall

WE HAVE learnt in an earlier chapter that Robert Hooke looked at a thick slice of cork tissue with his primitive microscope and saw that it was 'all perforated and porous much like a Honeycomb'. Surrounding the pores in the cork tissue were thick partitions which he called walls. The vast majority of the plant cells have these partitions and cytologists have continued to refer to them as walls.

The cell wall is an identifying character of the plant cells since the animal cells are devoid of it The plant cells are surrounded by a definite, rigid envelope, called the *cell wall*. To demonstrate the identity of the plasma membrane as distinct from the cell wall, place a group of cells in a concentrated solution of table salt The cytoplasm with its plasma membrane will shrink inside, while the cell wall remains in position.

Functions of the Cell Wall

The cell wall determines many features of a plant body It plays a major role in helping the aerial portion of the land plants to withstand gravitational forces. It is involved in the transport or movement of materials and metabolites in and out of the cell It counteracts physically the osmotic pressure produced by the cell contents. The cell wall plays an important role in cell expansion. Many enzymatic activities are also known to occur within the wall. Thus, far from being an inert secretion, the cell wall is a highly functional region outside the plasma membrane of the cell

Structure of the Cell Wall

The electron microscopic studies have shown that the cell walls are made of layers of crystalline microfibrils embedded within a formless matrix (Fig. 4.1). These microfibrils are composed of long-chain macromolecules. In bacteria and blue-green algae, these large molecules are largely proteins and polysaccharides. In most fungi, the mycelial wall is made up of a long-chain macromolecule known as chitin, a chemical that constitutes the chief portion of the exoskeleton in invertebrate animals. In green plants, it consists of bundles of long-chain cellulose giant molecules (Fig. 4 2). In woody plants, an encrusting layer of another long-chain macromolecule of lignin is deposited over the cellulose microfibrils. The properties of

hardness associated with wood arise from the cellulose cell walls impregnated with lignin

The microfibrils of the cell walls are embedded within a gel-like amorphous matrix. The matrix is composed of various polysaccharides, chiefly pectin and hemicelluloses. In the matrix of the cell wall we also find gums, tannins, resins, silica waxes, etc.

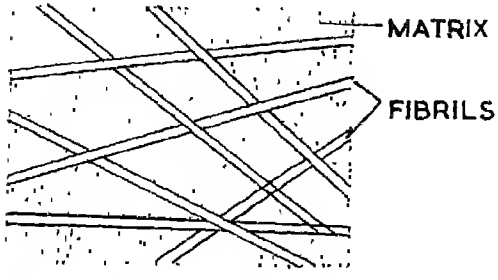


Fig. 4.1 Structure of the cell wall.

Plasma Membrane

Every cell is enclosed by a cell membrane. The membrane is also known as the plasma membrane. The plasma membrane forms an important barrier between the protoplasm and the outer environment of the cell. It is not only a protective cover, but it also plays a very important role since it determines which materials can flow into and out of the cell. The cell can remain alive as long as the cell membrane is able to discriminate and select what can enter or leave the cell. Hence, it is a living and dynamic membrane.

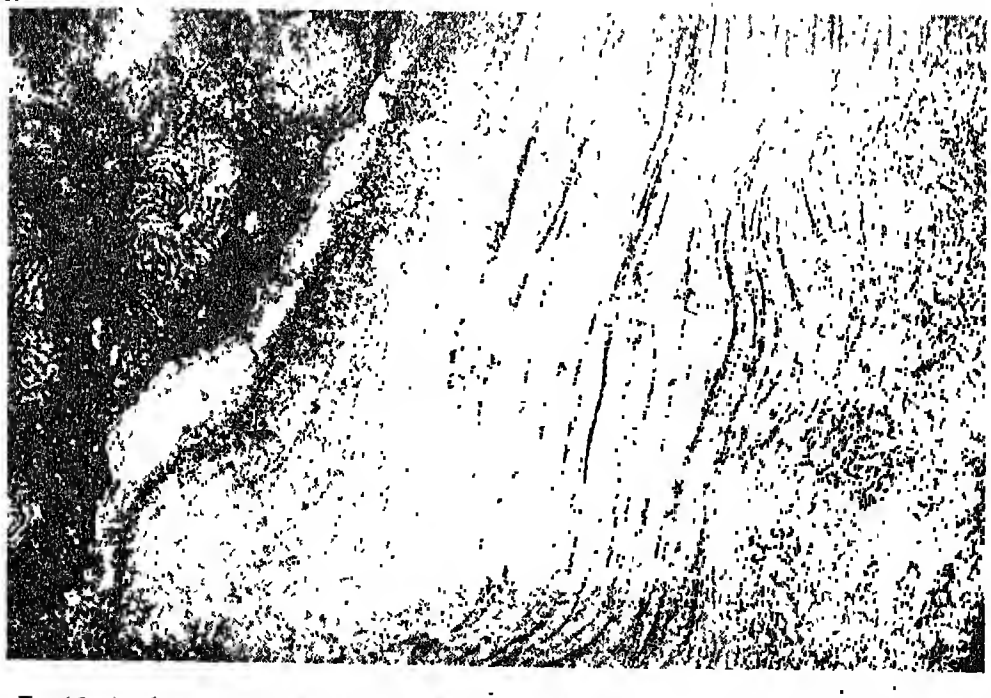


Fig. 4.2 An electron micrograph showing parallel bundles of cellulose fibers in the cell wall of wheat.

It may be interesting to know that we were able to learn a good deal about the structure and functions of the plasma membrane even before we could see it. We cannot see it since the thickness is below its resolving power. However, indirect information about the membrane was accumulated from physiological experiments. Now we are able to correlate its structure and functions not only because we can see it with the help of an electron microscope (Fig 4.3) but also because many other sophisticated techniques have contributed to our knowledge of the membranes. We can even isolate the membrane in pure form and study its properties. We can also prepare artificial membranes.

Structure of the Plasma Membrane

In 1935, James Danielli and Hugh Davson were the first to propose a molecular model of the plasma membrane (Fig. 4.4). From their physiological experiments they proposed that the plasma membrane consisted of three layers: a middle double molecular layer of phospholipids and two protein layers on either side of it. In effect, two protein layers sandwich the phospholipid bilayer. It was assumed that each

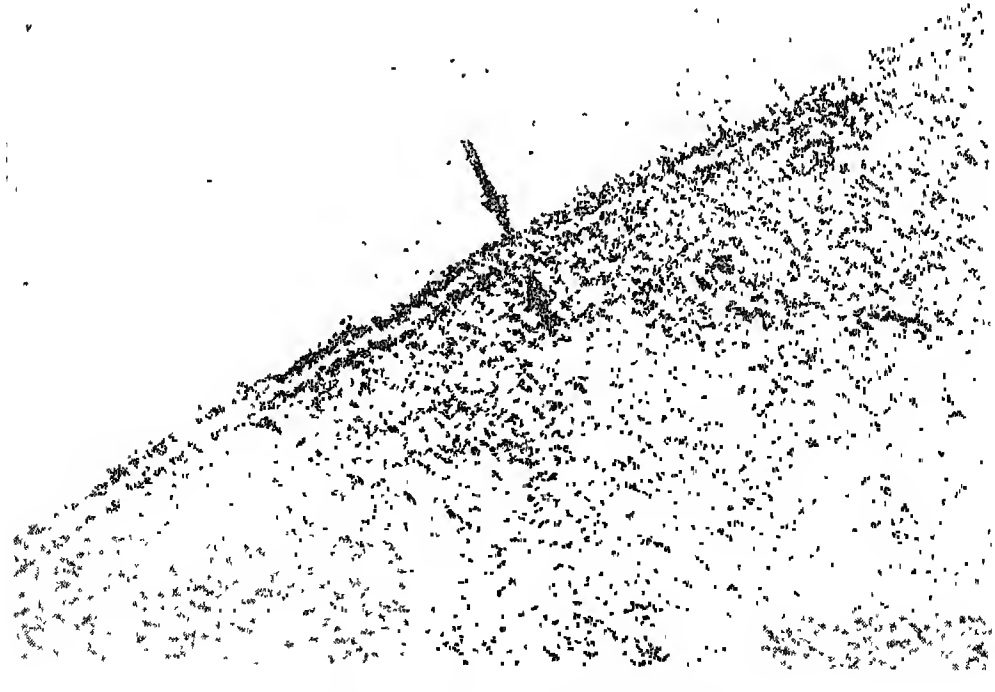


Fig. 4.3 Ultrastructure of the plasma membrane.

phospholipid had two ends: one hydrophobic and the other hydrophilic. The hydrophobic ends of phospholipid molecules faced each other in the interior while the hydrophilic ends were facing the outer protein layers.

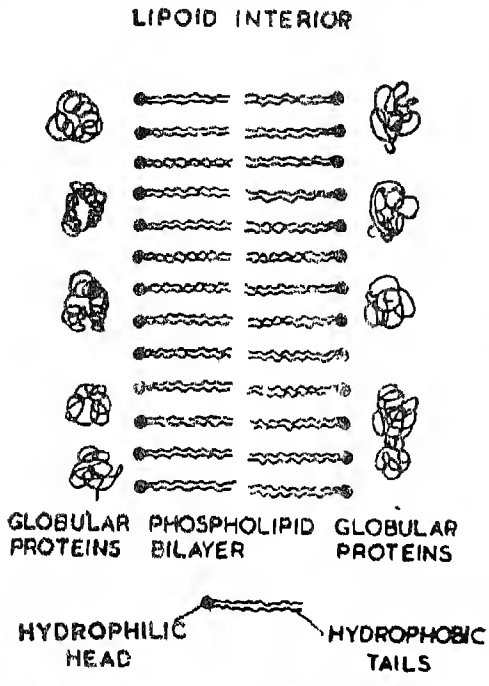


Fig. 4.4 Model of the plasma membrane — (After Danielli).

Later, J. David Robertson studied the plasma membrane of red blood cells with the help of an electron microscope and showed that the plasma membrane, indeed, had three layers of total thickness of 75 A° to 100 A°, each protein layer having thickness of 20 A° to 35 A° for the interior phospholipid bilayer. Robertson gave a concept of unit membrane (Fig. 4.5), meaning that all membranous structures of a cell have a similar structure of three layers and if there are more layers they are the multiples of the unit membrane. Robertson's model

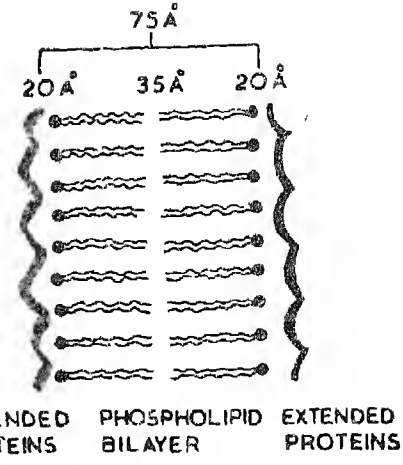


Fig. 4.5 Unit membrane — (After Robertson).

was widely approved but remained unsatisfactory, because it could not explain the dynamic nature and functional specifity of the membrane.

As more and more information was gathered, it became very clear that the three-layered structure was an oversimplified representation for many membranes. The unit membrane concept was also not valid since although membranes may have some features in common, they differed markedly in composition and functions in different cells and in different organelles.

In recent years, some more new models have been prepared Of these, the Fluid-Mosaic model proposed by Singer and Nicolson (Fig. 4.6) has found wide acceptance. According to this model, proteins do not always form a sandwich covering the entire hydrophilic surfaces of the lipid bilayers. Further, proteins play a very active role in the structure and functions of the membrane. According to this view, there are two categories of proteins: *peripheral* (or extrinsic) *integral* (or intrinsic). The integral proteins are tightly held in place by strong hydrophilic

or hydrophobic interactions (or both) and are difficult to remove from the membranes. The peripheral proteins are located superficially at the membrane and can be easily extracted. Some integral proteins may be present throughout the membrane while some are partly embedded in the lipid layers and partly projected on the surface. Many of these proteins — peripheral or integral — are enzymes. Some are known as *permeases* as they facilitate the entry of some substances. Different membranes of different cells and organelles differ in their protein and lipid compositions. The organization of lipids and proteins, as outlined above, imparts flexibility and specificity to the membrane.

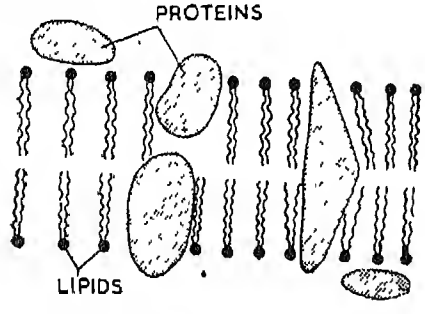


Fig. 4.6 Fluid mosaic model of the plasma membrane—(After Singer and Nicolson).

Transport Across the Membrane

Inspite of a lot of studies on the membranes, no satisfactory answer is yet available as to how exactly the molecules cross the plasma membrane. Several theories have been proposed but the exact mechanism is not yet known. It is believed that the transport of ions and molecules may involve both (*i*) passive and (*ii*) active transport. In passive transport, the molecules or ions move from high to low concentrations in a chemical gradient or an electrochemical gradient if the molecules are charged particles. The membrane plays a passive role as it allows simple *diffusion*. It is presumed that there are small pores, about 7 to 8 nm in diameter, to effect the passive transport. If the molecule concerned is that of water and moves from its higher to lower concentration through the membrane, the proces is called *osmosis*. The active process involves the movements of molecules even against the gradient, i.e., they may move from lower to higher concentration. Such active transport is supposed to involve either a carrier process or another energy-dependent process. In the carrier process, some kind of specific carrier (permease) is thought to facilitate the movement of the molecules across the membrane (Fig. 4.7). In this process, no expenditure of energy is involved. In the energy-dependent process, energy from the ATP is supposed to help speed up the transport of the molecules through the plasma membrane.

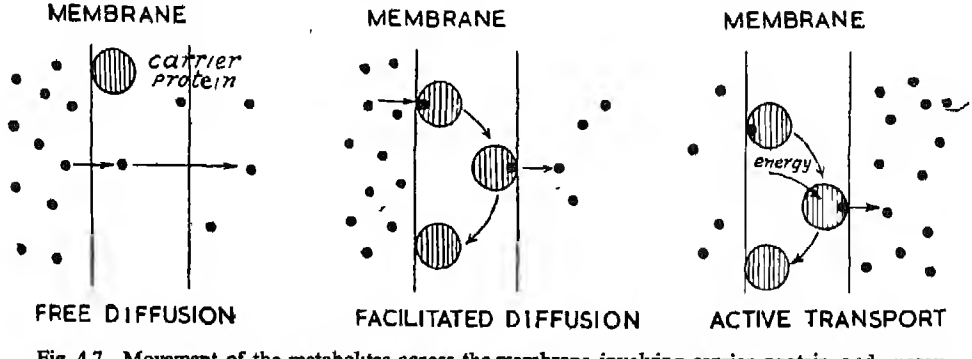


Fig. 4.7 Movement of the metabolites across the membrane involving carrier protein and energy.

The plasma membrane is too complex a structure and it is certainly very dynamic and flexible. Not only transport of molecules, ions, etc., take place, but exchange of specific molecules like those of *Na* and *K* may also occur. The molecules transported may be big or small. The processes outlined above may explain only partly the most complex nature of the transport mechanisms of the membranes. It is necessary to emphasize that the very existence of the cell depends on the cell membrane which is certainly very selective in allowing the entry and exit of the materials of the cell and it exerts great and subtle control over it. It is hoped that we will be able to know more about it since information is being gathered from many directions involving many different disciplines and techniques.

Pinocytosis and Phagocytosis

Some cells are required to ingest food or foreign bodies in bulk. For such materials, normal route of passage through the membrane is not possible. Hence, often in such cells the plasma membrane adopts special methods. The process by which substances in bulk are taken in by the plasma membrane is known as *endocytosis*, and the reverse process by which secretions or waste materials in bulk are thrown out by the cell is known as *exocytosis* The endocytosis involves *pinocytosis*, i.e., taking in (drinking) of liquid substances in a large amount and *phagocytosis*, i.e., engulfing of food materials or foreign bodies(Fig. 4.8). In pinocytosis or phagocytosis, the liquid globules or food particles get surrounded by an area of the plasma membrane which forms an invagination. Eventually, the invaginating ends fuse and pinch off to form a vacuole containing these substances. Such vacuoles later migrate towards the interior of the cell and merge with lysosomes and the material is thus digested.

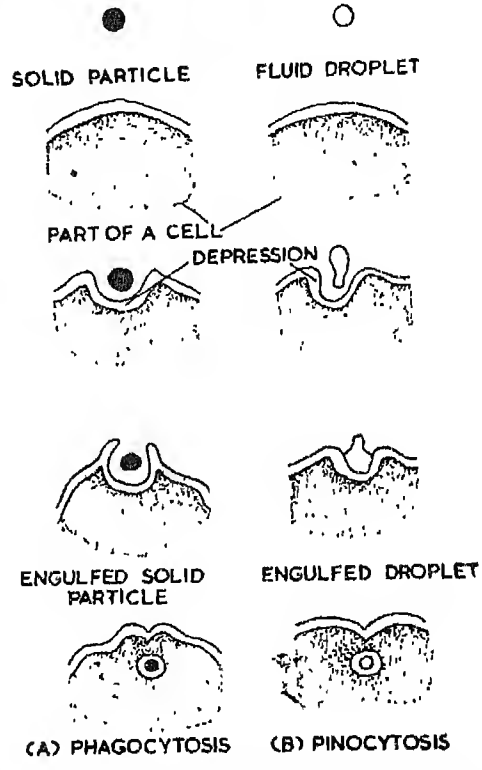


Fig. 4.8 Intake of solid and liquid materials by the cell — Processes of phagocytosis and pinocytosis

EXERCISES

1. Describe the structure of a typical plant cell wall.

2 Describe various structural models of the cell membrane. Which do you think is best related to its functions?
3. Describe how the cell membrane regulates the entry and exit of ions and molecules.
4. Explain the terms 'pinocytosis' and 'phagocytosis.'

CHAPTER 5

Endoplasmic Reticulum and Ribosomes

WE HAVE seen that the protoplasm enclosed within the cell membrane and separated from the outside (extracellular) world as well as from the nucleus, is known as *cytoplasm*. The cytoplasm was thought to be relatively homogeneous and was often referred to as hyaloplasm. One of the greatest contributions of the electron microscopy was to reveal an incredible complexity in the structure of the cytoplasm. With the help of the electron microscope it was demonstrated that in many cells of eukaryotes, an extensive membranous system — the *endoplasmic reticulum* (ER) — is observed in the cytoplasm.

The endoplasmic reticulum varies considerably in different cell types. In spermatocytes, it is in the form of only a few vacuoles. In cells engaged in lipid metabolism such as adipose tissue, it is quite simple in the form of a few tubules. However, it is quite extensively developed in cells active in synthesis, particularly in the synthesis of proteins and hormones, e.g., pancreas and liver cells. In the striated muscle, the endoplasmic reticulum takes a special form and is known as *sarcoplasmic reticulum*.

The endoplasmic reticulum exists as an extensive network of flattened sacs of *cisternae* (Fig. 5.1) which are formed by the encircling membrane sheet. In cross-section, it seems that the sacs are bounded by two membranes, each about 50 A° to 60 A° thick (Fig. 5.2). The endoplasmic reticulum may also be in the form of tubules or vacuoles.

Two kinds of endoplasmic reticulum are seen in cells: (1) smooth endoplasmic reticulum and (2) rough endoplasmic reticulum. When the regions of the endoplasmic reticulum are studded by granules of ribosomes on the outer face of the cisternae, the endoplasmic reticulum appears under the electron microscope as rough membranes and, hence, such endoplasmic reticulum is termed as *granular* or *rough endoplasmic reticulum*. In the endoplasmic reticulum if no such ribosomes are found attached to the outer surface, it is known as *smooth endoplasmic reticulum*. Apart from such morphological differences, the rough and smooth endoplasmic reticulums differ also in their functions. In the cells actively engaged in synthesising and secreting proteins, the rough endoplasmic reticulum is particularly highly developed; while in the cells which secrete and

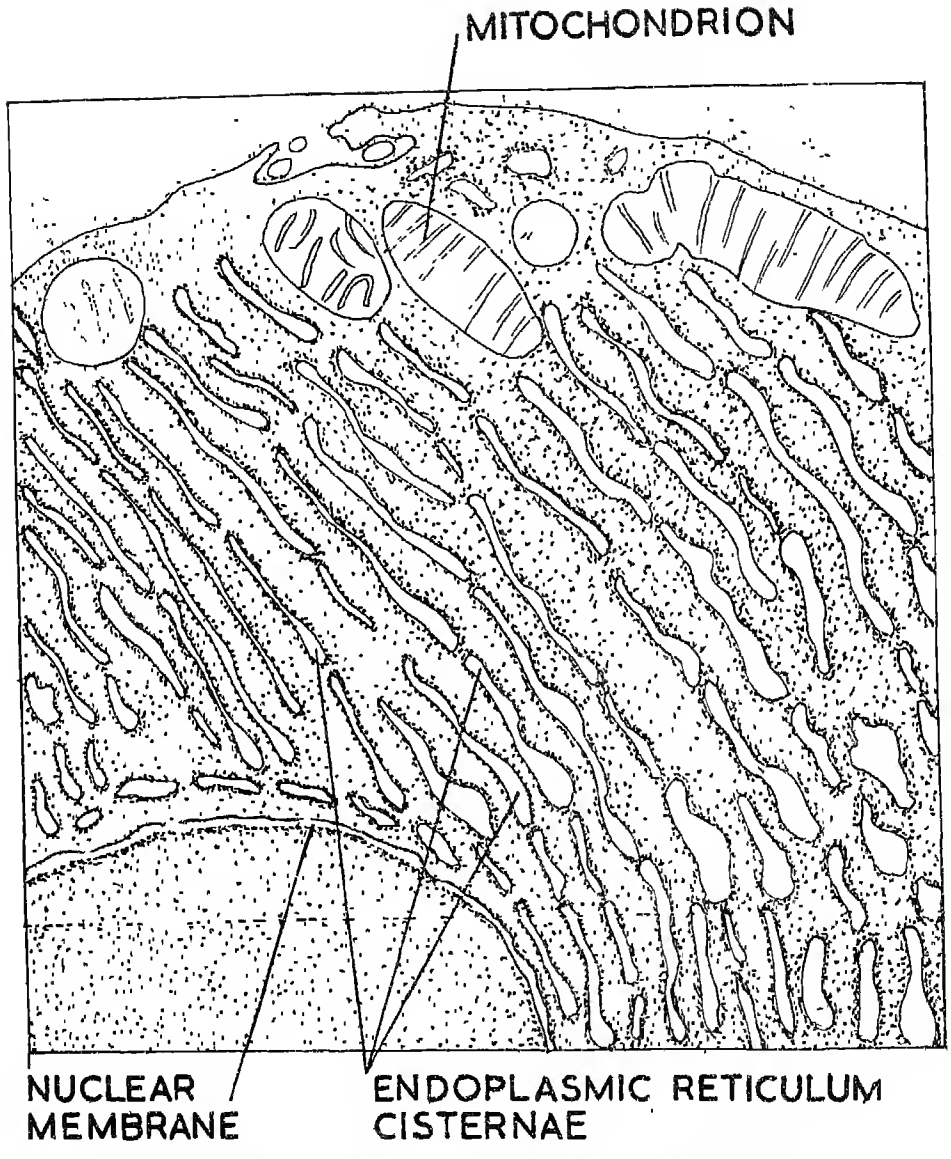


Fig. 5.1 Rough endoplasmic reticulum showing numerous flattened cisternae.

synthesise steroids, the smooth endoplasmic reticulum is well developed

The endoplasmic reticulum is much more than a passive channel for intracellular transport. It contains a large number of enzymes playing important roles in metabolic sequential reactions. Thus, polypetides are packaged into proteins. Proteins and lipids are complexed to form lipoproteins, and polysaccharides and glycogen are stored. All

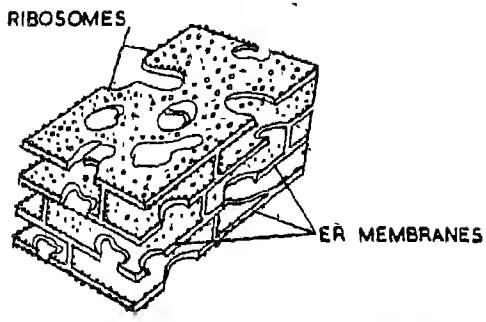


Fig. 5.2 Rough endoplasmic reticulum — a three-dimensional view.

these macromolecules are transported within and outside the cell through these vital channels of the endoplasmic reticulum.

It is not yet well known as to how the endoplasmic reticulum arises. However, it is belived that the expansion of the endoplasmic reticulum occurs through the synthesis of proteins and lipids necessary to form a new endoplasmic reticulum by the pre-existing reticulum.

Ribosomes

Ribosomes are essential for protein synthesis and are present in all plant and animal cells. In the electron micrographs, ribosomes are seen as spherical bodies, roughly about 150 A° to 250A° in diameter. Each ribosome consists of two distinct subunits of unequal sizes. Ribosome sizes are determined by the speed with which they sediment in a centrifuge, in terms of the Svedberg unit to measure the sedimentation co-efficient. In the cells of higher organisms, ribosomes of 80S are observed, while in bacteria, the ribosomes are slightly smaller, of 70S size. The 80S ribosome consists of 60S and 40S subunits, while the 70S ribosome consists of 50S and 30S subunits (Fig. 5.3). These subunits are further composed of smaller subunits. The ribosomal RNA molecules are large and they may account for almost 70 to 75 per cent of the total cellular RNA. Each subunit of a ribosome is a complexed ribonucleoprotein particle having roughly equal amounts of proteins and RNA. Not much is known about the ribosomal proteins, but it seems certain that there may be a large number of different types of proteins in each subunit of a ribosome. Some probably play structural roles, while others may have enzymatic functions. An enzyme, *peptidyl transferase*, which brings about the actual formation of a peptide bond, may be an integral part of the large subunits 60S and 50S.

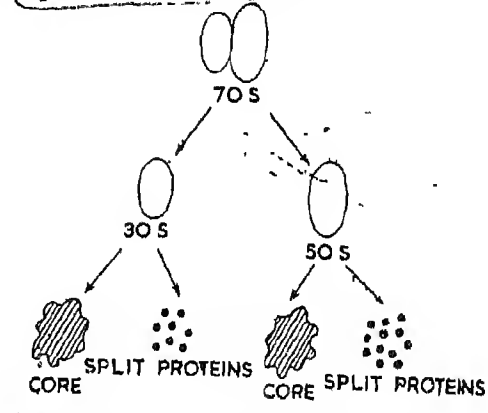


Fig. 5.3 Component subunits of 70S ribosomes.

The principal site of the synthesis of ribosomal RNA is the nucleous. It has been well established that the nucleolar DNA consists of the ribosomal genomes. Some precursor RNA is made on the nucleolar genes. The ribosomal proteins seem to be synthesised in the cytoplasm but migrate to the nucleoli for the formation of ribosomes by complexing with ribosomal RNA in the nucleoli.

Two distinct populations of ribosomes are recognised: those bound to the membranes and those that are free. Both play an important role in protein synthesis. Ribosomes which synthesise structural proteins leave the proteins synthesised in the hyaloplasm.

The bound ribosomes are known to synthesise globular functional proteins and transfer them to the cisternae of the endoplasmic reticulum.

During active protein synthesis, some ribosomes seem to occur in groups and are collectively known as *polyribosomes* (Fig. 5.4). Isolated polyribosomes consist of linear array of ribosomes interconnected by a strand of variable length, about 10 to 20 mm thick, which has been identified as mRNA.

Because ribosomes play an important role in protein synthesis, the structure and functions of each subunit and its components are of great interest.

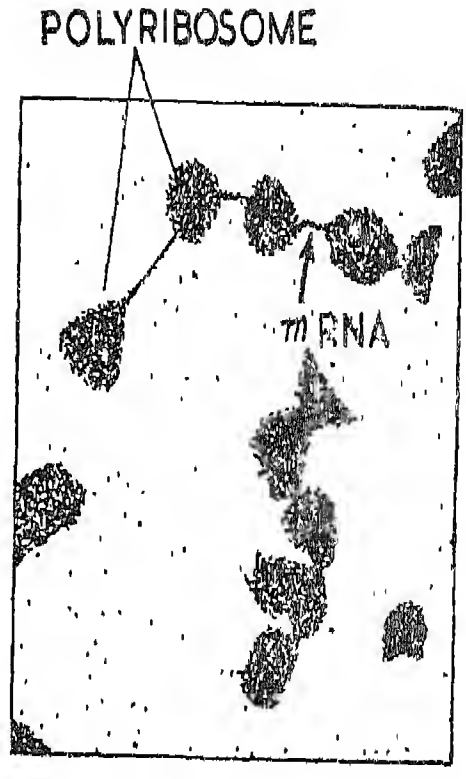


Fig. 5.4 Electron microscopic structure of polyribosomes (diagramatic).

EXERCISES

1. What are ribosomes? Mention their role in protein synthesis.
2. Mention the types of endoplasmic reticulum (ER) and state their functions?

CHAPTER 6

Golgi Apparatus

THE GOLGI apparatus was discovered in 1898 by an Italian scientist Camillo Golgi. He observed this structure in the nerve cell of an owl through the 'metallic impregnation' method. The technique was so drastic that, for long, many cytologists were not convinced of the very existence of the Golgi apparatus in cells and considered it to be an artifact. With the development of electron microscopy, the controversy was settled. All eukaryotic cells possess the Golgi apparatus, except a few cell types like red blood corpuscles of mammals.

Structure

It is difficult to observe and describe the structure of the Golgi apparatus through the light microscope, as it varies in shape, size and location. However, in the electron microscope, the structure appears as stacks of flattened sacs (cisternae), each bounded by a smooth-surfaced membrane. Often connected to the flattened sacs (cisternae) are vesicles of various sizes. There is great diversity in the size and shape of the Golgi apparatus in different cell types. In neurons, an extremely elaborate network surrounds the nucleus (Fig. 6.1). In the cells engaged in secretions of proteins, carbohydrates or hormones and in the absorptive cells, the apparatus is compact and is usually located between the nucleus and the cell surface where secretion or absorption takes place. In many plant cells, the Golgi apparatus appears to consist of many

Fig. 6.1 Golgi apparatus as observed in a nerve cell with the special staining method

unconnected units, called *dictyosomes* (Fig.6.2) Some plant cells may contain dozens while some seem dilated or inflated by materials accumulated in them. In most

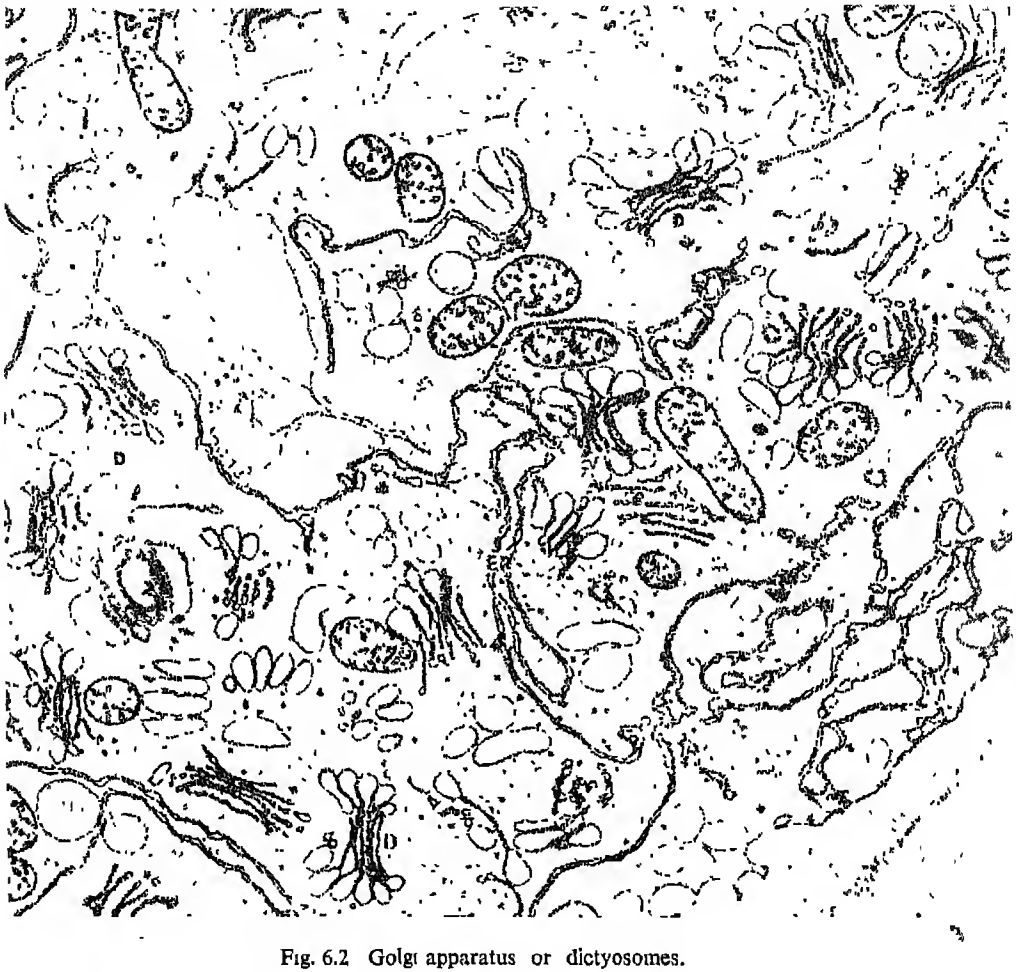


Fig. 6.2 Golgi apparatus or dictyosomes.

to hundreds of these dictyosomes, each of them being really a stack of cisternae.

The number of the Golgi bodies varies from three to seven in most animal cells, while in those of plants they may be ten to twenty. Some lower organisms may have a single sac. In the stacks, the sacs are separated from one another by a distance of about 200 A° to 300 A°. The width of the distance varies as some sacs appear uniformly flat, cells, the Golgi bodies are polarized and possess convex and concave surfaces as the sacs are concentrically bent towards the nucleus or outer surface (Fig. 6.3).

Functions

For some years it was thought that chemical synthesis did not occur in a Golgi body but it was believed to be a passive channel for materials synthesized elsewhere

in the cell. However, new evidences indicate that the Golgi apparatus is not a passive body but in fact it is metabolically very active and is also involved in the synthesis of some polysaccharides. It is also observed that linking of carbohydrates and protein in the formation of glycoprotein occurs in the Golgi apparatus. In the plant cells, the Golgi complex (dictyosomes) is known to synthesize pectin and some carbohydrates necessary for the formation of the cell walls and some secretions like mucilage, gums, etc. Several enzymes like *glycosyl transferase, thiamine pyrophosphatase* have been localized in the Golgi bodies. It is also involved in storage, condensation, packaging and transfer of materials. The packaging in a Golgi body involves wrapping of a membrane around a particular secretion and discharging it through the plasma membrane. In addition to their participation in processing cell secretions, the Golgi bodies also appear to be involved in membrane transformation, i.e., changing one type of membrane into another type. It is well established that secretory vesicles or primary lysosomes are produced from the sacs of the Golgi apparatus.

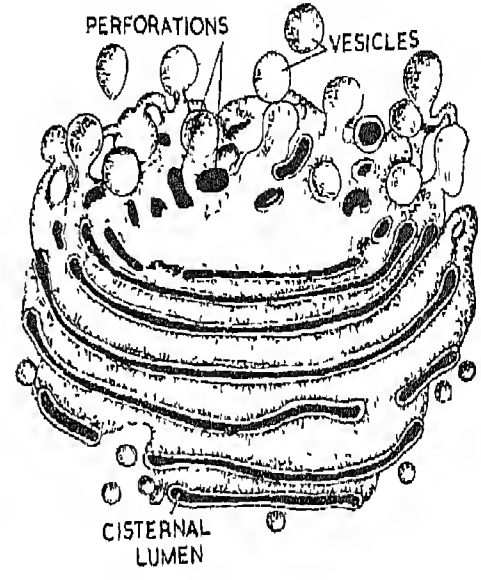


Fig. 6.3 Diagrammatic sketch of the Golgi apparatus.

EXERCISES

1. Describe the ultrastructure of the Golgi complex.
2. Mention the role of the Golgi complex in the cell wall formation.
3. Discuss the functions of the Golgi apparatus.
4. What are dictyosomes?

CHAPTER 7

Microbodies

SEVERAL CELL inclusions commonly found in cells are known as microbodies. There are many kinds of such microbodies, each having its specific roles and unique characteristics. We will discuss, here, lysosomes, peroxisomes and spherosomes which are the best, characterized microbodies.

Lysosomes

Historically, unlike other organelles, lysosomes were first studied by biochemical methods and were not seen under the electron microscope until about six years after the biochemical studies. Christian de Duve, a Belgian biochemist, is credited for discovering lysosomes in 1955 almost accidentally. He was trying to isolate from the rat liver certain enzymes which could hydrolyze carbohydrates. He found that activity of these enzymes was variable at all times when he homogenized the cells (Fig.7.1). He further found that older tissues gave a better yield of these enzymes and often several different hydrolyzing enzymes sedimented together in the same fraction which he isolated. Later, from the electron microscopic observation of these sedimated fractions, it was found that all these hydrolyzing enzymes were packed in

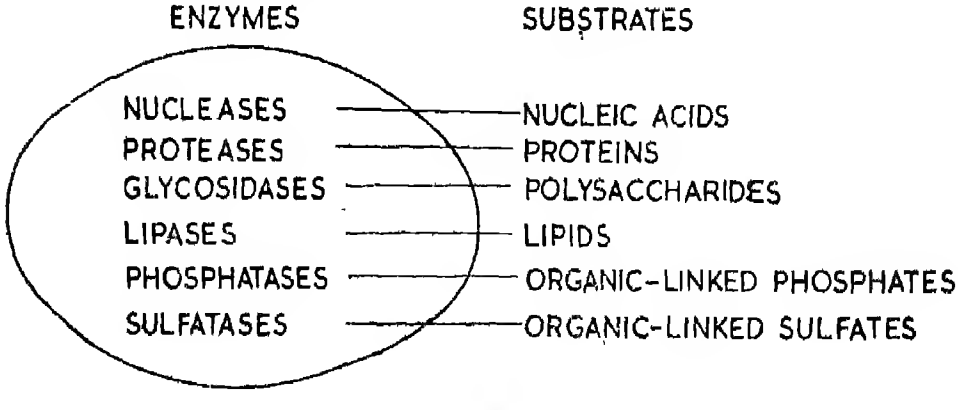


Fig. 7.1 Enzymes of lysosomes and substrates which they hydrolyze when the lysosome membrane breaks down.

small bodies which were called *lysosomes*. It has been shown that lysosomes possess a single membrane and contain nearly 40 different acid hydrolyses, one of the most important enzymes being the acid phosphatase. Lysosomes are comparatively small organelles, measuring, on an average, 0.5 μ in diameter.

Except for a few cell types, such as mammalian red blood cells (RBC), lysosomes probably occur in the cells of all protozoa and multicellular animals. Lysosomes have also been found in some kinds of plant cells, e.g., in yeast, fungi and green unicellular organisms such as *Euglena*.

All lysosomes are related, directly or indirectly, to *intracellular digestion*. The material to be digested may be of exogenous (extracellular) or endogenous (intracellular) origin. Collectively, the lysosomal enzymes are capable of hydrolyzing all classes of macromolecules in cells. The material on which the hydrolyzing enzymes act must enter the lysosomes since the enzymes remain confined within them (Fig. 7.2).

It is widely believed that the principal site of lysosome formation is the Golgi apparatus. The enzymes synthesized along the rough endoplasmic reticulum are transported through channels to the cisternae of the Golgi where they are packed into bodies which seem to bud off as lysosomes.

On the basis of morphology of contents and functions, the lysosomes are classified into the following four main types:

1. Primary lysosomes
2. Secondary lysosomes
3. Residual bodies
4. Autophagic vacuoles

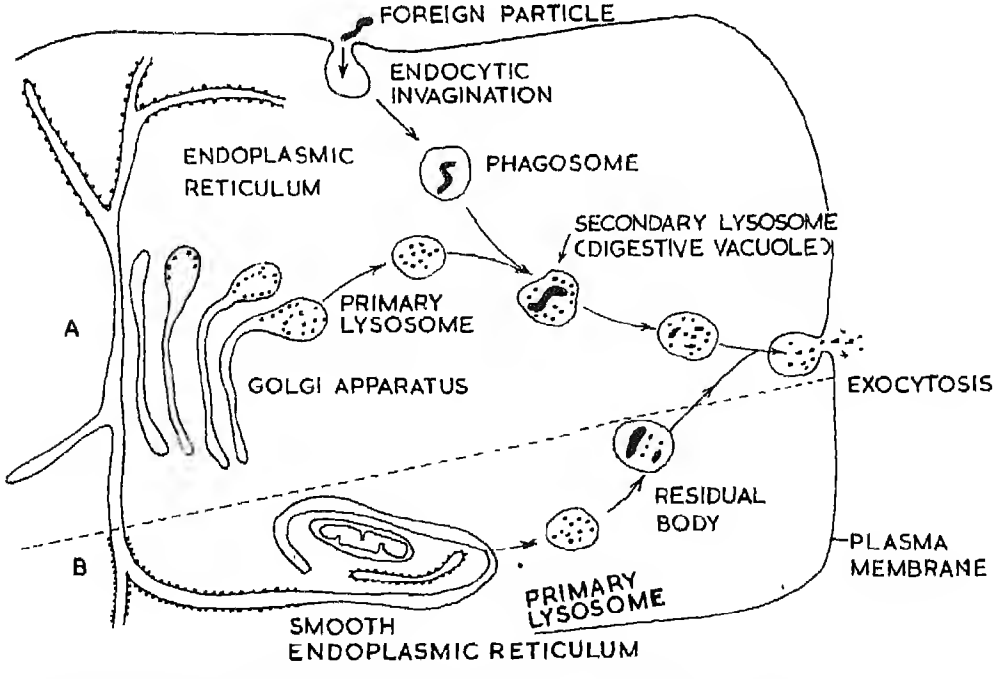


Fig. 7.2 Diagram of the stages of lysosome formation and intracellular digestion. A. Intracellular digestion. B Lysosomal activity during aging

Primary lysosomes are the bodies containing only enzymes. These are the ones produced from the Golgi apparatus. The enzymes are most likely inactive state in the primary lysosomes. When both the enzymes and the material to be digested or being digested are present within a lysosome, the lysosome is known as the *secondary lysosome*. The secondary lysosome may accumulate large quantities of undigested or indigestible molecules, the resulting structures are called *residual bodies*. On ageing and in certain pathological conditions, the lysosomes attack other intracellular organelles, surround them, envelop them in vacuoles and digest them. Such vacuolar lysosomes are referred to as *autophagic vacuoles*. For most types of digestions, the primary lysosomes often fuse with other vacuoles containing extracellular or intracellular materials in the cell to form the secondary lysosomes. Some important useful functions which lysosomes perform are as follow

1. They help in heterotrophic nutrition by intracellular and, under special conditions, also extra cellular digestion.
2. Lysosomes of leucocytes help in defence against cell infections by bacteria as well as microorganisms and guard against toxic molecules by digesting them.
3. Invasion by lysis of obstructing structures.
4. Under unfavourable starvation conditions, they help provide nutrition by cellular digestion.
5. They seem to be involved in fertilization, differentiation and metamorphosis.
6. They perform intracellular scavenging as part of the self-rejuvenation of long-lived cells and dead cells.
7. Programmed cellular breakdown associated with cellular ageing.

Peroxisomes

Peroxisomes, also known as microbodies, were first observed in the rodent kidney. They are distinctive organelles of widespread occurrance both in plants and animals (Fig. 7.3). They range from 0.5 to 1 μ in diameter and are delimited by a single membrane and contain a finely granular matrix. They often possess a central core called *nucleoid*. The nucleoid may consist of parallel tubules or twisted strands Peroxisomes are generally observed in close association with the endoplasmic reticulum. Peroxisomes in different plant and animal cells vary considerably in their enzymatic make-up, but they contain some peroxide-producing enzymes like urate oxidase, D-amino acid oxidase, B-hydroxyacid oxidase and catalase. Peroxisomes are somehow associated with some metabolic processes, e.g., photorespiration in the plant cells and lipid metabolism in the animal cells. However, their exact role still remains unclear.

Spherosomes

Spherosomes are bounded by a single membrane, contain enzymes and can be seen under the light microscope. However, their function is of less general nature than lysosomes. They show some affinity for fat stains, including the Sudan stains and even osmium tetroxide.

Spherosomes originate from the endoplasmic reticulum, arise by budding and contain enzymatic proteins capable of synthesizing oils and fats. Further development of spherosomes takes place through an increase in the lipid content with a concomitant decrease in protein.

The localization of enzymatic activity with spherosomes, and particularly with the acid phosphatase, has led to the suggestion that they are not essentially different from

lysosomes. But the specific lipidic nature of spherosomes indicates that these particles can be grouped morphologically and functionally as different from lysosomes.

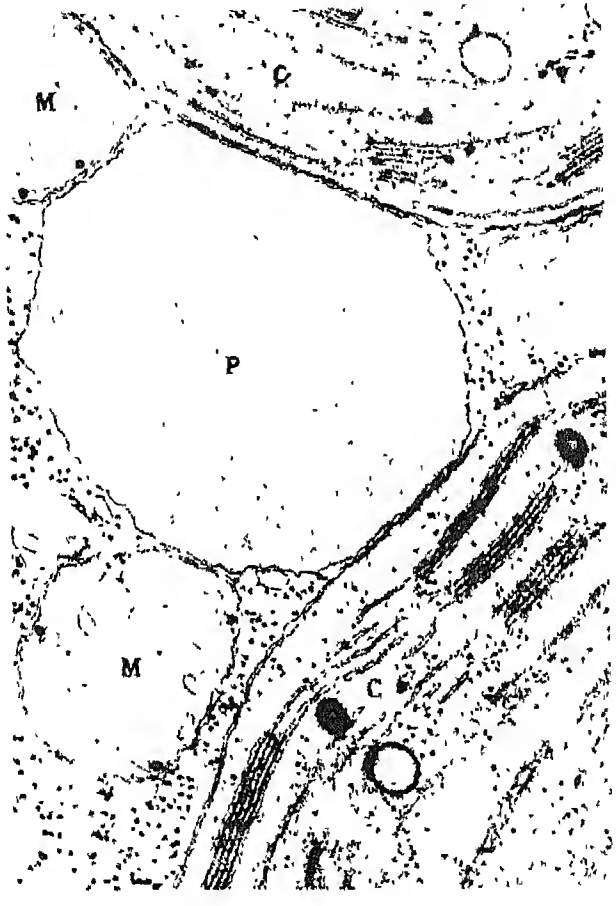


Fig 7 3 Peroxisome from a cell of grass. P Peroxisome. M. Mitochondia C Chloroplast

EXERCISES

1. Write an account of lysosomes and their role in cellular metabolism.
2. What are peroxisomes and spherosomes?
3. Why lysosomes are considered as suicidal bags?
4. Mention different types of lysosomes.

CHAPTER 8

Energy

ONE OF the most important facts of science is the principle of the *conservation of energy.* It states that energy is neither created nor destroyed, but can nevertheless be transformed from one form to another. All living organisms, in order to remain alive, need energy. This fact is known from another important physical law of entropy. This law states that all systems, living or non-living, if left to themselves, tend to increase the state of disorganisation and disorder (high entropy) unless free energy is provided. For living systems, the highest state of entropy, i.e. of disorganisation, is death. Hence, all living systems require a constant supply of energy to prevent death. Thus, energy must be available in living systems to power various processes of life. Table 8.1 shows how a cell utilizes its energy to perform some vital functions

TABLE 8.1

Cellular Energy	—Cell division
	—Synthesis of new constituents and molecules
	—Osmotic work
	—Transport of materials across the membrane
	—Nervous conduction
	—Muscular contraction, etc.

Chemical energy is the most suitable form of energy for living systems, since it can be easily *transferred, transformed* and *stored.* Although chemical energy is often converted into other forms of energy, it is the primary energy found in all living cells.

The energy currency of the living cells is a chemical compound called *adenosine triphosphate* (ATP). ATP consists of a nitrogenous base, adenine, linked to the five-carbon sugar, called ribose (Fig. 8.1) A string of three phosphate molecules is linked to the sugar molecule. A phosphate group has one atom of phosphorous and three of oxygen.

Most of the energy of the ATP molecule is in the bonds of the two phosphate groups at the end. When an ATP molecule reacts with water with the help of an enzyme, the bond between the second and the third phosphate is broken. Energy is released that can be measured as heat energy.

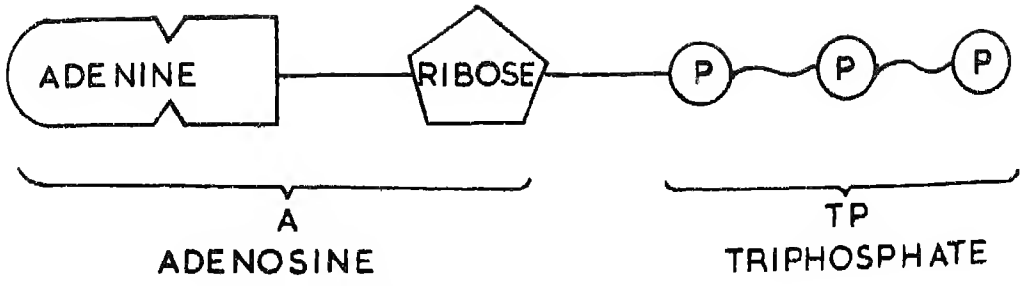


Fig. 8 1 Diagrammatic representation of ATP-molecule.

ATP + $H_2O \rightarrow ADP + P_i + 30K$ joules of energy–Removal of the terminal phosphate residue from ATP thus releases 30 kilo joules of heat/mole. Hence, the bond is called the energy-rich phosphate bond. Inside the living cells, the energy released is not lost as heat, but it is used to perform cellular functions When a molecule of ATP gives

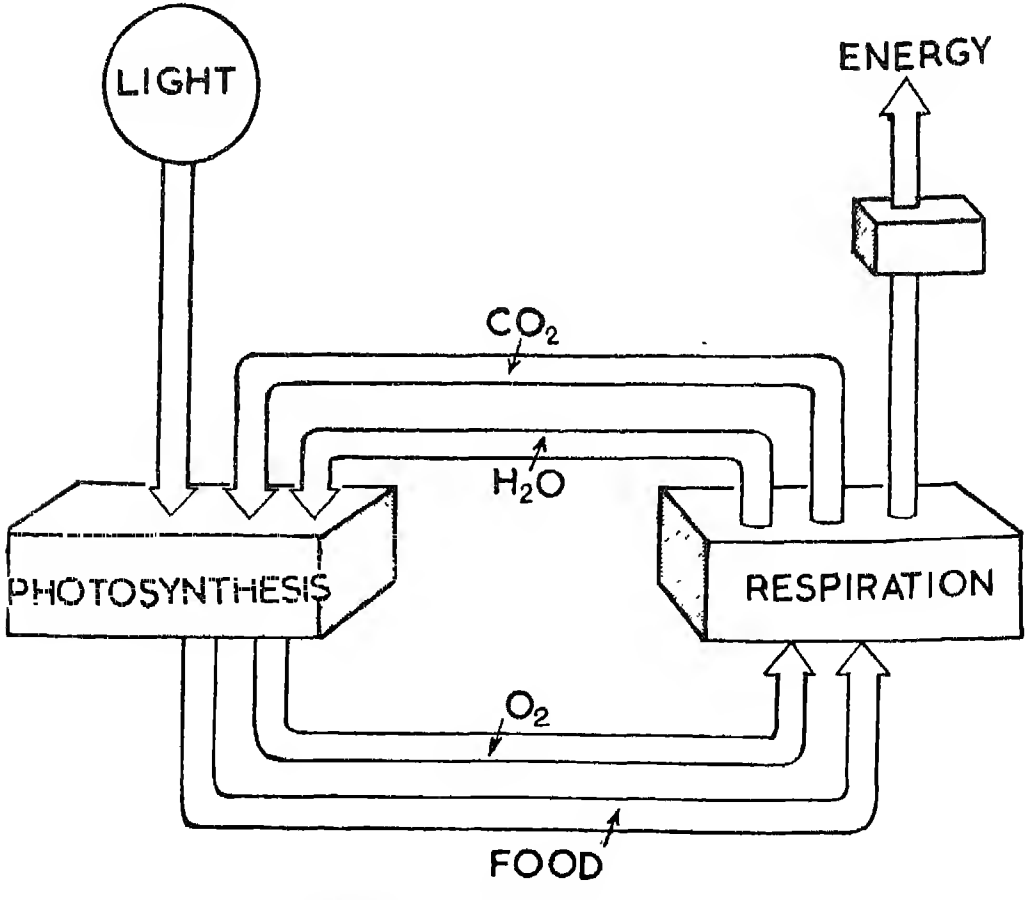


Fig 8.2 Diagram showing the energy-flow in the living world.

up one energy-rich phosphate group, it becomes adenosine diphosphate (ADP). To form a molecule of ATP again, ADP must combine with one phosphate group. The energy required to link the phosphate group with ADP is supplied by the breakdown of organic compounds, e.g., glucose within the cell.

The Sources of Energy in the Cells

As mentioned above, living organisms obtain energy for the synthesis of ATP by breaking down organic compounds such as sugars, fats or amino acids. However, the primary source of energy is the sun. The light energy from the sun is utilized by green plants through a process of photosynthesis to synthesize sugars from carbon dioxide and water. Animals, in general, depend on plants for the organic compounds in the form of food which is utilized for production of energy, i.e., ATP through the process of respiration Although carbon dioxide, water and oxygen are cycled between respiration and photosynthesis, energy flows in one direction in the living world (Fig. 8.2). Energy is converted from sunlight by photosynthesis and released by respiration.

In the living cells, these functions of respiration and photosynthesis are performed by two special organelles, mitochondria and chloroplasts, respectively We will now study these organelles and how they perform these functions.

EXERCISES

1. Give several reasons for the fact that energy–releasing reactions occur in a number of steps.
2. Which substance would you take for a quick supply of energy–glucose or sucrose ? Why ?
3. Mention the vital functions of a cell where energy is utilized.
4. Why chemical energy in the form of ATP is the most suitable form of energy for cellular processes ?

CHAPTER 9

Mitochondria

MITOCHONDRIA are known as the 'power-houses' of the cell, since they are energy-generators. Mitochondria were first observed by Altman in 1886 and were called *bioblasts*. Benda (1897) stained them and studied in some detail and he named them *mitochondria* (Greek. *mito*—thread, *chondrion*—granule) Mitochondria are present in all plant and animal cells, with the notable exception of bacteria and blue-green algae. [illegible] as it may elucidate the possible evolutionary origin of mitochondria Some highly specialized cells like mammalian erythrocytes (RBC) have lost their mitochondria as a secondary feature Their number may vary from a few per cell to several thousand, depending on the type and functional state of the cell.

Microsterias, an alga, contains only one mitochondrion, while an amoeba, *Chaos chaos*, may contain as many as 50,000. A human liver cell may contain about 1000, while a kidney cell may have 300 to 400 mitochondria Although their sizes vary considerably in different cell types, diameters of 0 5 to 1.0 microns and lengths of 5 to 10 microns or more are common. The size, shape and number may vary according to the physiological, pathological and differentiation states of the cells. By suitable stains, mitochondria can be easily observed in a thin tissue section They can also be seen in the living cells with the help of the phase-contrast microscope.

Structure

Under the light microscope, the mitochondria of a cell are observed as filamentous, spherical or sausage-shaped bodies (Fig. 9.1). The detailed structure of a mitochondrion is revealed by the electron microscope. A mitochondrion possesses a double membrane —an outer membrane and an inner membrane. The outer membrane is separated from the inner membrane by a space 60 A° to 100 A° wide. The inner membrane is extensively infolded. These infoldings are called *cristae*. The cristae are the unique identifying feature for mitochondrial profiles in the electron micrographs. Due to the extensive infoldings, the total surface area of the inner membrane is much greater than that of the outer membrane. Enclosed by

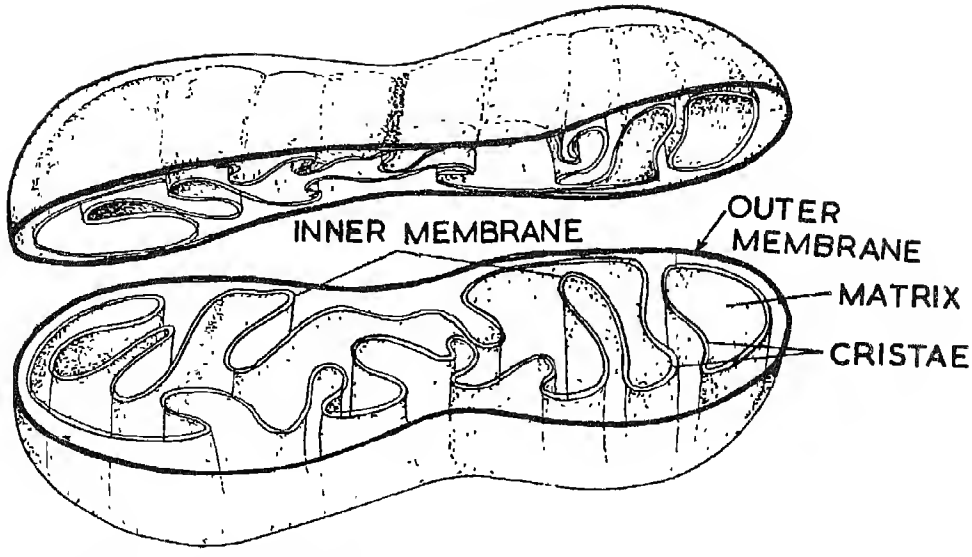


Fig. 9.1 Diagrammatic structure of the mitochondrion.

Fig. 9.2 A part of the mitochondrial cristae showing an elementary particle.

the inner membrane is the *matrix*. By a special technique of negative staining in the electron microscopy, the inner membrane (cristae) is observed to possess the tennis racketlike numerous stalked bodies. They are known as *elementary particles* or *oxisomes* (Fig 9 2). Now, techniques are available by which we can not only isolate mitochondria in pure form, but we can even obtain separately the outer and inner membranes as well as the matrix Thus, each part of a mitochondrion can be obtained for biochemical analysis. Mitochondria contain approximately 25 to 35 per cent lipid, 5 to 7 per cent RNA and traces of DNA, 60 to 70 per cent protein Nearly 60 different enzymes have been found to exist in a mitochondrion It is now possible to localize some of these enzymes in the various parts of a mitochondrion and relate them to the functions they perform.

Functions

Mitochondria perform the main functions of conversion and transfer of cellular energy, through synthesis, storage and release of ATP for use in cellular activities, and governing the transport of materials and water in and out of their own membranes. Thus, mitochondria are miniature biochemical factories which produce energy-rich ATP molecules from food-stuffs, oxygen and ADP. The following diagram (Fig. 9.3) explains the inputs and outputs of a mitochondrion,

Although the process of production of ATP, as described above, appears so simple, it involves a large number of interrelated reactions, each driven by a specific enzyme. The conversion, release and transfer of energy is a stepwise gradual process

The process of phosphorylation, i.e, linking of phosphate to ADP, requires energy which is obtained by an orderly controlled release and capture of electrons. In chemical terms, oxidation and reduction reactions involve transfer of electrons from one molecule to another. The molecule losing electrons is *oxidised* and the one gaining electrons is *reduced*. The energy obtained by such transfer of electrons from one molecule to the other is utilized to attach phosphate molecule to ADP, i.e., to make ATP This process is known as *oxidative phosphorylation* This transfer of electrons is harnessed and controlled by a series of reactions which utilize oxygen to couple it with hydrogen atoms which, in turn, are released as side-products of the electron transfer system. The coupling of hydrogen with oxygen results in the formation of water and the chain of reactions involved in this process is known as *respiration* Thus, oxidative phosphorylation is linked up with respiration for the production of ATP molecules. The stepwise release of electrons occurs through a cycle of

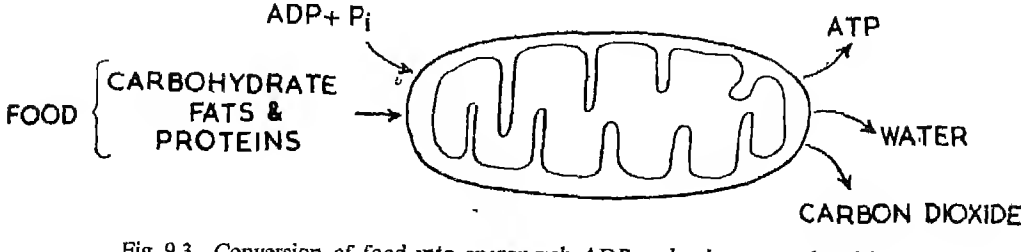


Fig. 9 3 Conversion of food into energy-rich ADP molecule in mitochondria.

metabolic events collectively known as the *citric acid cycle* or Krebs' cycle, since it was first described by a scientist named Krebs. All the food-stuffs are degraded to a compound known as acetate which combines with Co A and initiates the citric acid cycle. When the whole cycle is completed, for example, from glucose molecule as a fuel, three molecules of carbon dioxide, 30 molecules of ATP and one molecule of water are produced.

The precise correlation and co-ordination of all these reactions require much co-ordinated structural organization. We now know that while some enzymes are located in the outer membrane, some in the space between the membrane and the matrix, most of the Krebs cycle enzymes are located in the inner membrane, and the final step of phosphorylation of linking phosphate to the ADP molecule occurs in the elementary particle which contains an enzyme ATPase for such reaction.

Biogenesis of Mitochondria

Three general hypotheses have been proposed to explain how new mitochondria are formed: (1) *De novo* (formed a new) from precursors in the cytoplasm, (2) from other non-mitochondrial membranes, such as the nuclear or plasma membrane, (3) by growth and division of the pre-existing mitochondria. The existing evidences favour the third possibility of the origin of mitochondria, while the first two have enjoyed less favour and the weakest of all is the first possibility With the discovery of DNA in mitochondria and the presence of their own ribosomes, it is most likely that mitochondria arise out of other pre-existing mitochondria through their own synthetic machinery. However, nuclear control in such a process is not ruled out.

EXERCISES

1. Mitochondria are sometimes called the power-houses of the cells. Why?
2. Describe the ultrastructure of a mitochondrion
3. Explain the process of phosphorylation.
4. How are new mitochondria formed ?

CHAPTER 10

Chloroplasts

CHLOROPLASTS are the most important of all the organelles of the cells Although they are found mostly in the cells of green plants, all living organisms directly or indirectly depend on them for obtaining energy. They are the basic life materials which are capable of harnessing the light energy from the sun to synthesize food substances All the greenery of the earth is due to chloroplasts Chloroplasts are cytoplasmic organelles found in the plant cells. Only a few plants like fungi and some bacteria are devoid of them. A chloroplast is a form of plastid. There are several kinds of plastids However, they can be grouped mainly into three types. the leucoplasts which are colourless plastids; the chromoplasts which contain colours other than the green, and the green-coloured plastids known as chloroplasts.

Chloroplasts are relatively large organelles and can be readily visible under the light microscope even in unstained preparations as they themselves are green in colour They vary greatly in size and shape from species to species. In some algae, they are cup-shaped or ribbonlike spirals and fill a large part of the cells. In higher plants, many chloroplasts are present in each cell, generally as ovoid, lens-shaped or disclike bodies In grass leaf cells, there may be as many as 50 to 60 chloroplasts in each cell. In higher plants, they measure 2 to 4 by 5 to 10 μ in size. A typical chloroplast is composed of 50 to 60 per cent proteins, 25 to 35 per cent lipids, 5 to 10 per cent chlorophyll, 1 per cent pigments other than chlorophyll, and small amounts of RNA and DNA.

Structure

Chloroplasts (Fig.10.1) are bounded by two membranes, about 300 A° in total thickness. The outer membrane is similar to the plasma membrane. The inner membrane is very intricately elaborated to form a system of lamellae The inside of the chloroplasts is clearly divisible into two parts: (1) the embedding, colourless ground substance *stroma*; and (2) the membrane system made up of closed flattened sacs called *thylakoids* Thylakoids are closely packed into certain areas, like piles of coins known as *grana*. These may be as many as 40 to 60 grana per chloroplast and each granum may consist of 2 to 100 coinlike thylakoids.

Fig. 10.1 Ultrastructure of a chloroplast from the corn leaf

D — Membranes S — Stroma
G — Grana R — Reticulum

Thylakoids can assume a variety of configurations in different species of plants. It can be simple parallel sacs running lengthwise, or may be in a complex interconnecting network of the sacs (Fig. 10.2) The other conspicuous feature of chloroplasts is the presence of some starch granules which often accumulate near a special region known as *pyrenoid* in algae.

Functions

The most important function of chloroplasts is *photosynthesis* In the process of photosynthesis, radiant energy from the sun is utilized by the chlorophyll molecules to produce chemical energy and synthesize organic compounds like sugar for food. Thus, photosynthesis involves actually two main processes (1) photophosphorylation, i.e., formation of ATP molecules with the help of radiant energy, and (2) utilizing this energy (ATP) to couple carbon dioxide and water to synthesize glucose. An oxygen molecule is also evolved in the process of photosynthesis. The first reaction is light dependent and cannot occur in dark. Hence, [illegible] The other [illegible] n light and can occur even in dark. It is known as *dark*

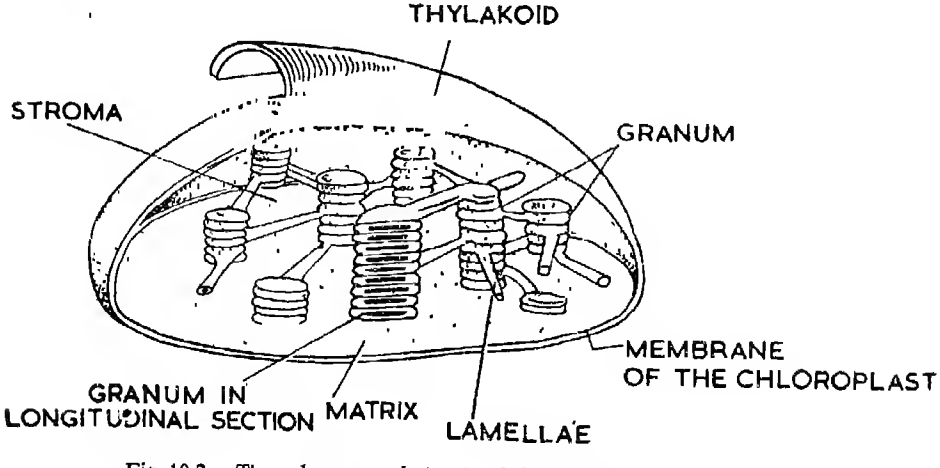


Fig. 10.2 Three-dimensional structural diagram of the chloroplast.

reaction. We have already learnt details of these processes in Class XI.

EXERCISES

1. Distinguish between the light reactions and dark reactions of photosynthesis.
2. Describe the structure of a typical eukaryotic chloroplast.
3. What are different types of plastids ?
4. Can we consider photosynthesis as a fundamental process of life ?

CHAPTER 11

Centrioles, Cilia and Flagella

CENTRIOLES appear as small granules associated with a mitotic spindle during the cell division Centrioles were observed to occur in pairs situated at the polar ends of a mitotic apparatus In typical non-dividing cells, there is one pair often located close to the Golgi body. Centrioles are cylindrical structures, approximately 0.15 μ in diameter and 0.3 to 0.5 μ in length. This is just the limit of resolution of the light microscope and, hence, very little was known about their detailed structures until they were examined under the electron microscope

When seen under the electron microscope, centrioles have a very characteristic appearance (Fig 11.1). When they occur in pairs, each lies perpendicular to the other. Each centriole is made up of nine sets of tubular structures arranged in circular fashion Each of the nine sets is a triplet composed of three microtubules. Each microtubule has diameter of about 250 nm. These triplets are embedded in an amorphous matrix Sometimes, delicate strands appear to connect sets of the triplets to each other and other fine fibrils can often be seen radiating from the central core of the cylinder, presenting in sections a configuration similar to the "Cartwheel" The cartwheel structures are not seen in all centrioles. In the longitudinal section, the cylinder is seen as a heavy-walled structure with a denser proximal end.

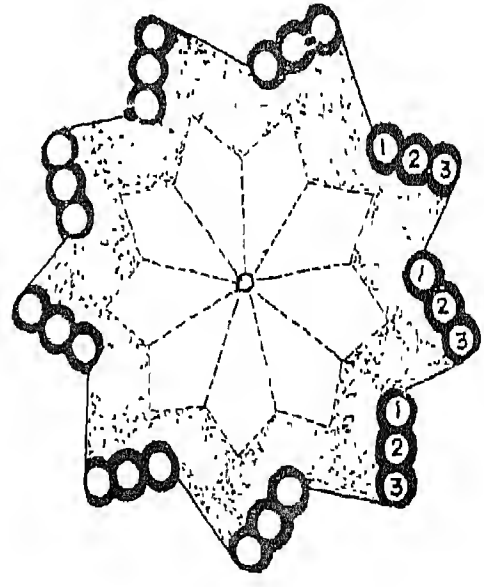


Fig. 11.1 Diagram of the centriole structure in cross-section showing nine subfibre-triplets and the giant cartwheel pattern of five fibres that is sometimes present

Centrioles are found in all eukaryotic cells, except in amoeba, red algae, pines

and all flowering plants where flagellated cells are absent. Centrioles are involved in division. Similar structures when they produce flagella or cilia, they are called *basal bodies* Basal bodies have the same fundamental nine sets of triplet organization

Basal bodies appear to be formed *de novo* in the cells It has been shown that like mitochondria and chloroplasts, they also contain DNA and are self-perpetuating semi-autonomous structures. They may also contain some RNA.

Cilia and Flagella

Cilia and flagella are specialized surface structures which in most cases serve as propellers in locomotion of the cells. In the cells, which are stationary, they serve other purposes like elimination of particles or driving food or water currents Flagella are long whiplike appendages and may be as long as 150 μ, while cilia are short, with an average length of 5 to 10 microns. Cilia occur in large numbers in a cell, while flagella are usually very few, only one or two per cell The diameters of both cilia and flagella are less than 0.5 microns.

Cilia and flagella are structurally similar (Fig. 11.2) and arise from basal bodies which are like centrioles The electron microscopy has shown that cilia, flagella, basal bodies and centrioles structurally resemble each other in having nine sets of tubules arranged in a cylinder. Unlike centrioles, an additional pair of tubules is found in the centre of the cylinder and the tubules are "doublets", instead of "triplets", of centrioles. Thus, the pattern of organizations of cilia and flagella is 9+2, instead of 9+0, like that membrane, whereas cilia and flagella are bounded by a membrane which is an extension of the plasma membrane.

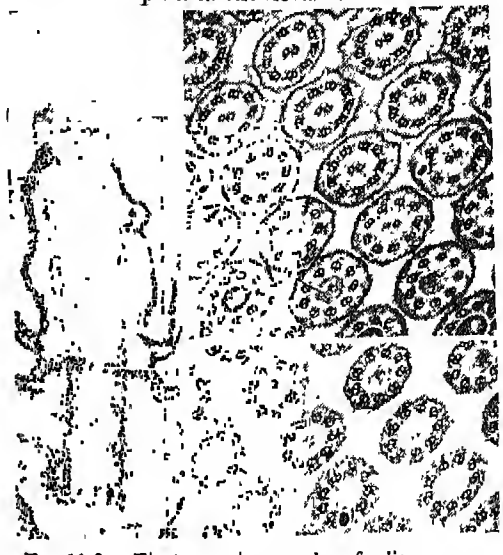


Fig. 11.2 Electron micrographs of cilia.
A — Longitudinal section. B and C — Cross-sections
C Centriole or basal body DP - Ciliary plate. CP - Cilium.

The biochemical analysis of cilia and flagella has shown that the peripheral microtubules contain a special type of protein called *tubulin*, somewhat similar in nature to the *actin* of the muscle fibre. It is believed that a sliding mechanism similar to the mechanism of muscle contraction is involved in the movement and bending of cilia and flagella to effect locomotion of the cells.

The establishment of structural and functional similarities of widely different organelles like cilia, flagella, basal bodies, centrioles and sperm tails is one of the great achievements of modern biology.

EXERCISES

1. Describe the structures of centrioles and basal bodies.
2. Mention the main functions of centrioles.
3. State the difference between flagella and cilia.
4. Compare the ultrastructure of a cilium with that of a centriol

CHAPTER 12

Interphase Nucleus

A CELL which is not in the process of division is known as the interphase cell. Every eukaryotic cell at interphase contains a highly specialized region — a nucleus The cells without nuclei cannot survive long The mammalian red blood cells live only for few months since they do not possess nuclei. Cells or unicellular organisms like *Amoeba* or *Acetabularia* from which nuclei were experimentally removed were unable to survive for long unless new nuclei from other such cells were transplanted. Thus, nuclei are essential for the survival and long-term continuation of the cells. Further, the cells without nuclei cannot undergo regular division and cannot differentiate. Hence, the interphase nucleus has three main functions: cell maintenance, cell replication and control of the cytoplasmic activity. Most of these functions are asserted because the nucleus contains DNA and produces all the cellular RNA necessary for protein synthesis.

The major chemical composition of the nucleus is 9 to 12 per cent DNA, 5 per cent RNA, 3 per cent lipid, 15 per cent basic proteins and about 65 per cent other proteins. Most of the RNA made in the nucleus rapidly moves out into the cytoplasm Nuclei also contain some enzymes, such as polymerases, for the synthesis of DNA and RNA.

The nucleus is separated from the cytoplasm by the nuclear envelope. Within the nucleus at interphase (Fig. 12 1), there is heterogeneous distribution of chromatin material. The chromatin material includes euchromatin and heterochromatin. During the cell division, rodlike bodies, called chromosomes, are formed by tight coiling of the chromatin fibres. The euchromatic state of chromatin is the uncoiled state, while the heterochromatin state is a little more compact coiled state of chromatin and, hence, gets darkly stained if stained with proper dyes.

The chromatin fibre can be considered as a basic structural unit. A metaphase chromosome of eukaryotes as well as some typical chromosomes like polytene chromosomes found in the salivary gland cells of some flies and lampbrush chromosomes found in oocytes of amphibians can be considered as morphological variants associated either with the state in the cell cycle or the state of differentiation of cells.

It is now well established that a chromatin fibre consists of a continuous linear DNA duplex strand associated with basic proteins—*histones*, *non-histone* acidic or neutral proteins, a small amount of RNA and some enzymes such as DNA and RNA *polymerases*. How all these are involved in a chromatin structure and function is not yet very clear, but currently a lot of work is being done on this aspect.

Another most important component of the nucleus is *nucleolus*. Every cell nucleus may possess one or more nucleoli. The nucleolus is a rounded body without a surrounding membrane. It is especially

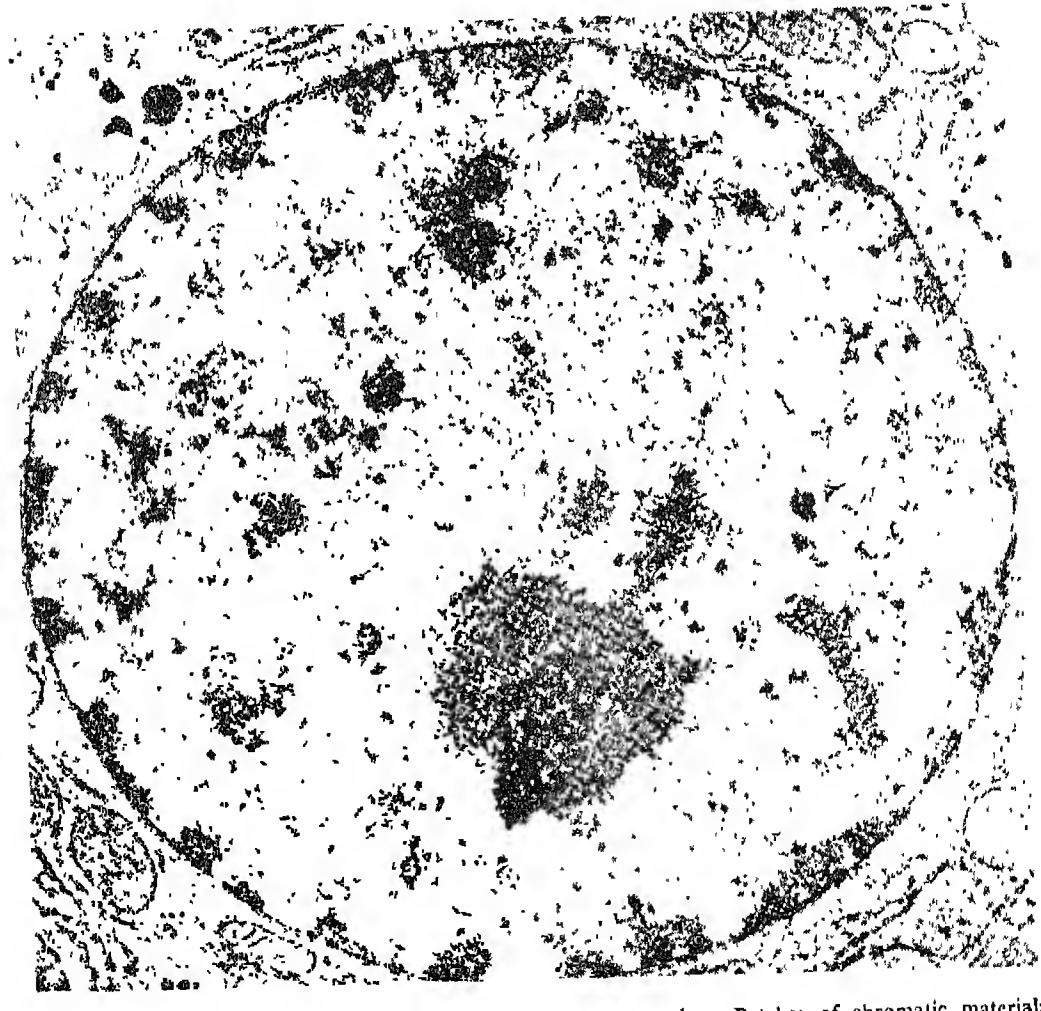


Fig. 12.1 Photograph showing the ultrastruture of interphase nucleus Patches of chromatic materials are seen.

rich in RNA and proteins It is now well established that nucleoli contain DNA whose main function is to form precursor RNA for the formation of ribosomes. Some chromosomes have specific sites, called nucleolar organiser regions, which give rise to the nucleoli during the interphase In electron micrographs, nucleolus shows at least two different zones—granular and fibrillar.

The structure of the 'nuclear envelope' is of great interest for nuclear-cytoplasmic interaction Under the electron microscope, the nuclear envelope appears as a flattened sac, more like the membrane of the endoplasmic reticulum. The outer surface of the nuclear membrane may even contain ribosomelike granules, while the inner surface is smooth. One of the distinctive features of the nuclear envelope is that it often contains a large number of pores (Fig.12 2A) The pores may be roughly circular or polygonal, having diameter of about 500 A° to 800 A° (Fig 12 2B). The pores are not simply holes in the membranes since substances do not pass through them easily although they may represent areas of macromolecular exchanges between the cytoplasm and the nucleus.

During the cell division, the membranes of the nuclear envelope break up into fragments. It has been suggested that some of these fragments are re-used in the formation of new nuclear envelopes in the daughter nuclei after the cell division.

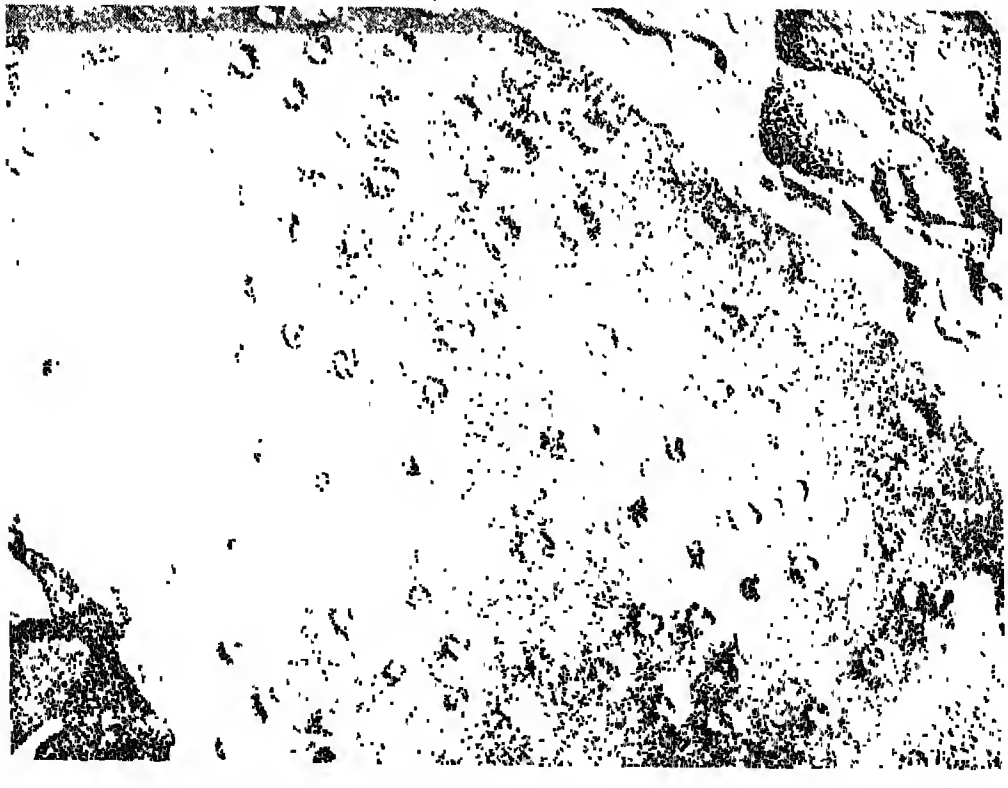


Fig. 12 2A Electron micrograph of the nuclear envelope showing numerous pores

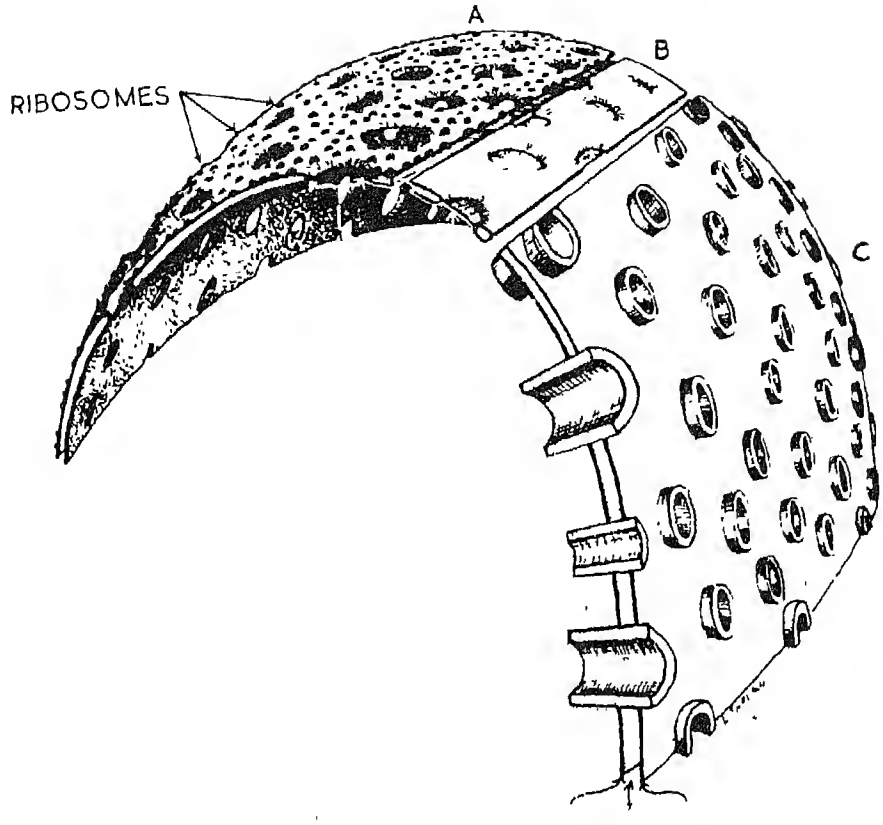


Fig 12.2B Diagrammatic sketch of the nuclear envelope. It shows the upper surface with ribosomelike particles and different kinds of pores

In the prokaryotic cells like bacteria, there are no well-formed nuclei possessing nuclear membranes. In the prokaryotic system transcription and translation form a continuous process. In the eukaryotic cells somehow in evolution the processes of transcription and translation have been separated in two different compartments—the nucleus mainly for the purpose of transcription, while the cytoplasm performing the function of translation (refer to chapter 16). Although the nucleus is the organelle controlling most of the cytoplasmic activities, it cannot exist by itself. The cytoplasm and the nucleus must interact in order to maintain the integrity and life of the cell.

EXERCISES

1. Describe the role of the nucleus in the nucleocytoplasmic interaction.

2. Discuss the ultrastructure of a nuclear envelope.
3. Describe the structural organization of a typical eukaryotic chromosome.
4. Discuss the functions of a nucleolus.
5. Explain how the so-called resting stage—interphase—is the most active stage of the cell cycle.

CHAPTER 13

Enzymes and Regulation

WE ARE familiar with the baker's yeast. It is an aggregate of single cells. Yeast can thrive well in a medium containing only glucose. But the yeast cell is not all glucose. It is constructed of many kinds of molecules, most of which are far more complex than glucose, e.g., fats and proteins. Hence, it is obvious that the yeast cell somehow knows the secret of transforming glucose into some other molecules. Indeed, one of the greatest triumphs of biochemistry is the unravelling of this secret by which glucose is chemically converted into a large variety of molecules necessary to construct and maintain the cell integrity. These chemical changes do not occur in a single step; instead, the process is like an assembly-line as shown below:

$$A \xrightarrow{\textit{enzyme 1}} B \xrightarrow{\textit{enzyme 2}} C \xrightarrow{\textit{enzyme 3}} D$$

These stepwise reactions are under the regulation of organic catalysts called *enzymes*. An enzyme is a protein which can enhance the efficiency of a biochemical reaction Each step in the synthesis or degradation of a molecule is catalyzed by a specific enzyme. In fact, life functions are not possible without enzymes.

In the case of enzymes, the overall function depends not only on the kind and amount of enzymes present but also on their activity. Activity is determined by many factors, including the presence of a substrate, product, hormone and some other molecules. The activity of enzymes may vary from one-cell type to another. We will study some of these in this chapter.

Chemical Nature of Enzymes

All the enzymes so far purified and crystallized are proteinous in character. Some enzymes do have a metallic or non-metallic component, prosthetic group, but protein forms by far the great bulk of an enzyme. Proteins are made up of amino acids, of which there are 20 different kinds. Each amino acid molecule has a carboxyl (COOH) group and an amino ($-NH_2$) group. The carboxyl end of one amino acid unites with the amino end of another to form what is known as a *peptide bond*. Thus, many amino acids can link together to form dipeptides, tripeptides and

polypeptides. A protein molecule is composed of one or many peptide chains. A protein molecule has at least 200 to 300 peptide linkages. Most of the enzymes are large molecules containing hundreds of amino acids and more than one peptide chain.

The peptide bonds holding the amino acids together in a particular sequence constitute the primary structure of proteins. Portions of the peptide chains of some proteins prove to be twisted into a helix, a structure that is probably stabilized by the hydrogen bonding from one amino acid to another, in turn, below it. Such a helical arrangement constitutes the secondary structure of these proteins. The individual peptide chains are further extensively coiled into spherelike shapes with the hydrogen bonds between the amino and carboxyl groups and various other kinds of bonds cross-linking one chain to another and stabilizing the tertiary structure. It is presumed that the protein shape is almost completely determined by the kind and arrangement of the amino acids present, because the coiling and folding must result when these come into proper contact at points where cross-bonding can occur. Proteins function in living organisms both as enzymes and as structural elements. The ability of proteins to carry out specific reactions is the result of their primary, secondary and tertiary structure.

Some enzymes require another organic substance in the medium in order to function properly. In a few cases, enzymes actually consist of two molecular parts. One of these is protein, called *apoenzyme*. The other molecular part is a smaller, non-protein molecule. This smaller molecule is called *coenzyme*. Its name signifies that it works with the main apoenzyme molecule as a co-worker in bringing about a reaction. The two molecular parts in this case are chemically bonded to each other. In other cases, the *coenzyme* is combined only briefly with the enzyme. In either case, the presence of the *coenzyme* is needed before any catalytic activity takes place. *Coenzymes* may also work by removing one of the products of the catalyzed reaction. A chemical analysis of the smaller coenzymes has shown that they often contain a vitamin as part of the molecule. This has led to the idea that some vitamins serve as coenzymes. This would explain why the absence of certain vitamins causes extensive physical defects in the organism. The enzyme which works with the vitamin-based coenzyme cannot work by itself. Therefore, an entire series of important physiological reactions may be blocked. It also explains why only a small supply of vitamins is sufficient to fulfill the requirements for good health. Like enzymes, coenzyme molecules must be replaced only from time to time, at a relatively slow rate.

Mode of Action

Enzymes combine with substrates before yielding the products of the reactions they catalyze. In other words, the enzyme and substrate form an intermediate complex before decomposition of the substrate can occur (Fig. 13.1). It is a two-step reaction as follows:

1. Enzyme + substrate = enzyme substrate complex
2. Enzyme substrate complex = enzyme + product substance

There are also certain enzymes which merely join two molecules together.

How do enzymes work ? The existence of the very phenomenon of specificity argues for the fact that the enzyme must be

combining with the substrate molecule in order to act. Presumably, this combination works as lock and key (Fig. 13 2A). If the right key fits into the right lock, the lock can be opened, otherwise not. Of particular importance is the idea that molecules have specific geometric shapes (Fig. 13 2B). Proteins are able to act as enzymes primarily because their shape provides surface configurations into which other molecules can fit. The molecules which are acted upon by the enzymes are called substrates of the enzymes. It can be seen that only a substrate molecule with the proper geometric shape can fit into the active site of the enzyme. However, under certain conditions some other molecules very similar to the substrate may also combine with the active site of an enzyme. In such an event, such molecules may compete with the substrate and the reaction may slow down or stop. Such substances are called *competitive inhibitors*, since they act to prevent the production of a substance (Fig. 13 2C). There is a good deal of experimental evidence to support the idea that enzymes do work in the manner indicated by the lock-and-key analogy.

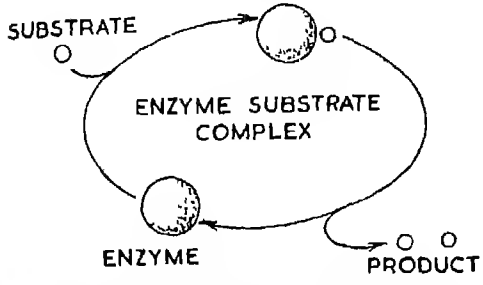


Fig 13.1 Formation of enzyme-substrate complex during enzyme action

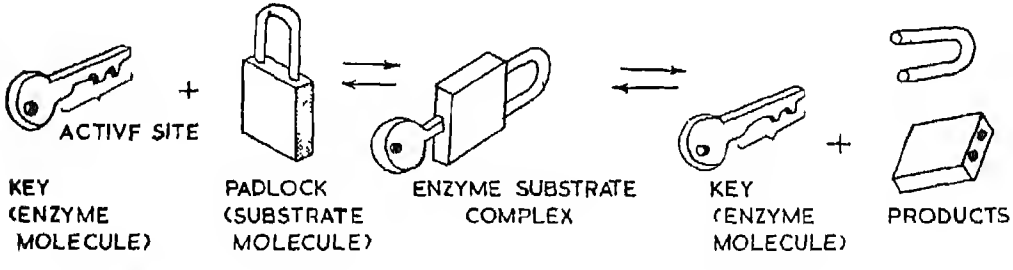


Fig. 13 2A Key-and-lock model to understand enzyme-substrate interactions.

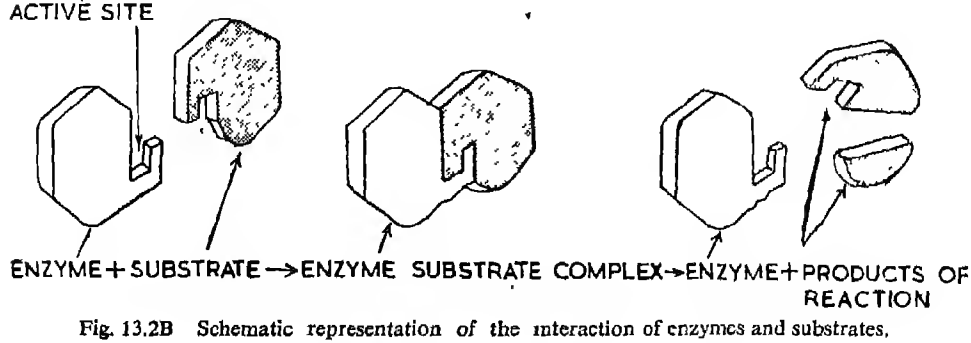


Fig. 13.2B Schematic representation of the interaction of enzymes and substrates.

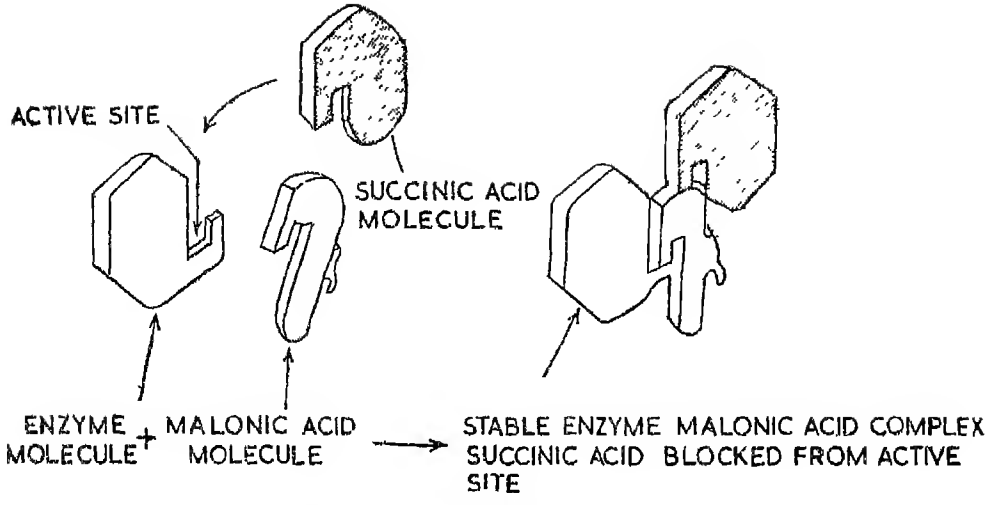


Fig 13.2C Diagrammatic representation of competitive inhibitions.

Nomenclature and Classification

Different systems of classifying enzymes are known but the recent and widely accepted classification is based on their chemical activity. Individual enzymes, except for a few, and groups of enzymes are named by adding the suffix *ase* to the name of the substrate on which they act. There are *hydrolases* which hydrolyze larger molecules into smaller ones. *Proteinases* break up proteins into smaller amino acid molecules, *amylases* break up starch into sugar, *sucrase* breaks up sucrose into glucose and fructose, *lipases* break up fats into glycerol and fatty acids, and *nucleases* break up nucleic acids into nucleotides.

The suffix *ase* indicates that the compound is an enzyme. Other enzymes, such as trypsin, end with *-in*. This signifies that they, like all enzymes, are proteins. Enzymes ending in *-in* were discovered and named before an international ruling was made in favour of the *-ase* ending However, few enzymes have been renamed, e.g., salivary enzyme ptyalin is now called salivary amylase. Enzymes are also named after the compounds they attack. Thus, peptides are attacked by peptidases; peroxides by peroxidases; lipids by lipases; ester linkages by esterases; hydrogen atoms are removed by dehydrogenases, and so on.

Factors which Affect Enzyme Activity

Temperature

Being proteins, enzymes may be completely denatured at high temperatures. In other words, at a certain temperature, an enzyme carries out its catalytic activity in such a way as to allow the reaction being catalyzed to proceed most rapidly. This should not be taken to mean that the faster a chemical reaction proceeds, the more efficient the reaction is. Efficiency in this case refers to the number of collisions the enzyme is making with the molecules of the substance on which it carries out its activity.

The effect of a slight temperature change upon the activity of an enzyme brings up a point of considerable biological significance. An organism's metabolic chemistry may speed up or slow down as a result of a slight

change in body temperature. Some animals, such as mammals, maintain a fairly constant body temperature despite great fluctuations in the external environment. Therefore, their metabolic rate is relatively independent of the external temperature. Other animals, such as fishes, amphibians and reptiles, have body temperature which rises and falls in close correlation to the outside temperature. Such animals must remain inactive during certain times of the year when the temperature falls below a certain level. Furthermore, they may even die if the temperature, as an enzyme inactivating factor, rises to a certain point.

Hydrogen ion Concentration (pH)

Changes in pH can cause denaturation of the enzyme molecule.resultinginthereduction of activity. However, this does not appear to be the major effect of pH on the enzyme catalyzed reactions. Typically, an enzyme will have an optimum pH, but a shift to the alkaline or acid side causes impairment of the activity. We can also infer that different enzymes have different pH optima (Fig. 13.3).

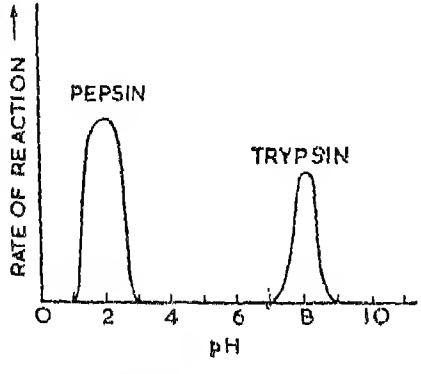


Fig. 13.3 A graph showing pH dependency of the enzymes.

Enzyme Concentration

An increase in the enzyme concentration would increase the rate of reaction. If sufficient substrate is present, doubling the enzyme concentration usually causes a two-fold increase in the rate of reaction.

Product Concentration

New substances arise by the interaction of the enzyme-substrate complex, but with the increase in the product concentration, the rate of reaction falls because the enzyme must be freed to combine with the other set of molecules.

Substrate Concentration

An increase in the substrate concentration will increase the number of molecules in the immediate vicinity of the enzyme's active sites and, as a result, increase the chance of a substrate molecule coming in contact with an active site.

Isoenzymes

It was believed that only one enzyme could act on a given substrate However, it is now realized that enzymes slightly different in molecular structure can also perform identical activities. Such enzymes are known as *isoenzymes*.

There are more than 100 enzymes which are known to exist as isoenzymes. One of the best known examples of isoenzymes is *lactic dehydrogenase* (LDH) which catalyzes the reaction of pyruvate to lactate. There are five or more LDH isoenzymes which slightly differ in their physical properties and amino acid sequences. The relative proportions of these isoenzymes are characteristic of each tissue and of each stage of its differentiation.

Regulation

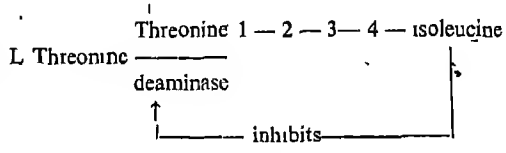
One of the striking characteristics of living (organisms) systems is that they function in a coordinated manner despite their high degree of complexity. One wonders at the masterly regulation of the multitude of reactions that go on simultaneously in a cell How is such precision achieved ? What are the controlling mechanisms ? Mainly, there are two types of such control mechanisms operating within a cell : one at the enzyme level where the enzymes, substrate and product themselves are involved in such regulatory process, and the other at the genetic level where the genes regulate the enzyme production.

At the Enzyme Level

It is well known that when certain metabolites accumulate in a cell, they inhibit their own production. This kind of control mechanism is known as *feedback inhibition.* This control mechanism is analogous to a refrigerator thermostat which regulates temperature by turning the switch off and on in response to fluctuations in the temperature inside the refrigerator

The completion of overall biochemical reactions in living cells involves a number of intermediate reactions As few as two or three or as many as thirty or forty reactions may make up a series leading to the synthesis or degradation of a specific compound, since all these steps are enzymatically controlled. If even one of these enzymes is affected, inhibited or destroyed, the entire series of reactions will be affected This may bring about far-reaching effects and even the death of the cells, the tissues or the organism itself.

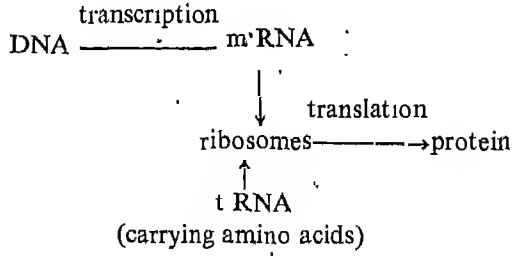
An excellent example of such a control has been demonstrated in *Escherichia coli* in the synthesis of amino acid-isoleucine. When the threshold level of isoleucine accumulates in the cell, the bacterium stops its further synthesis. It was found that the excess of isoleucine inhibits the activity of the enzyme called threonine deaminase which catalyzes the first step of the reacton chain leading to the production of isoleucine. This type of metabolic control, where the first enzyme of a sequence is inhibited by the end-product, has been termed *end-product inhibition* or, more technically, *feedback inhibition.*



This type of control is similar to the automatic feedback circuits used in most of the electronic devices

At the Genetic Level

DNA is the master molecule which controls the synthesis of proteins as shown in the following diagram.



We know that proteins are the end-product of the gene function. This enables us to work backward to pinpoint the gene An average-sized protein molecule contains approximately 500 amino acids. For the selection requiring of each of these, a triplet

of three bases is required. Thus, for such a protein, the gene is a portion of the DNA molecule containing 1 500 base pairs

With the evidence firmly supporting the replication of all the genetic material during the cell division, it is evident that each cell of a living organism contains the same complement of genes. The question then arises: What makes those cells different?

It seems evident that some selective mechanism must control the genes, allowing some to function and restraining the others. The growth of a plant or an animal is basically determined by its mechanisms for switching the genes on and off in orderly sequence.

How protein synthesis is controlled was suggested by two French scientists, Jacob and Monod, who were awarded the Nobel prize for their postulation. They proposed that a set of *structural* genes (e.g , A, B, C, etc) are controlled by an *operator* gene O (Fig. 13.4). When the operator is switched off, no mRNA is formed, and no protein synthesis takes place. When the operator is switched on, RNA is transcribed in the DNA and protein synthesis begins. This switching off and on of the operator is controlled by a *regulator* gene, R. The signal from the regulator to the operator is made by means of a substance called *repressor*, which is produced by the regulator. The repressor combines with the operator and shuts off the transcription, thereby the system remains in the switch-off position. However, when certain metabolites, the *inducer*, are present, they combine with the repressor and prevent it from inactivating the operator. In this situation, the genetic system returns to the switch-on position and protein synthesis proceeds.

The gene control system may also be operative in multicellular organisms. The proteins of chromosomes in eukaryotes seem to be the regulators of the gene action. It is not unlikely that other kinds of regulator substances may also exist. The chromosomal proteins, in turn, may be under the control of small molecules like auxin and hormones, coming from outside the cell.

Thus, we can well understand that metabolic processes in living systems are very complex, yet each process is regulated and

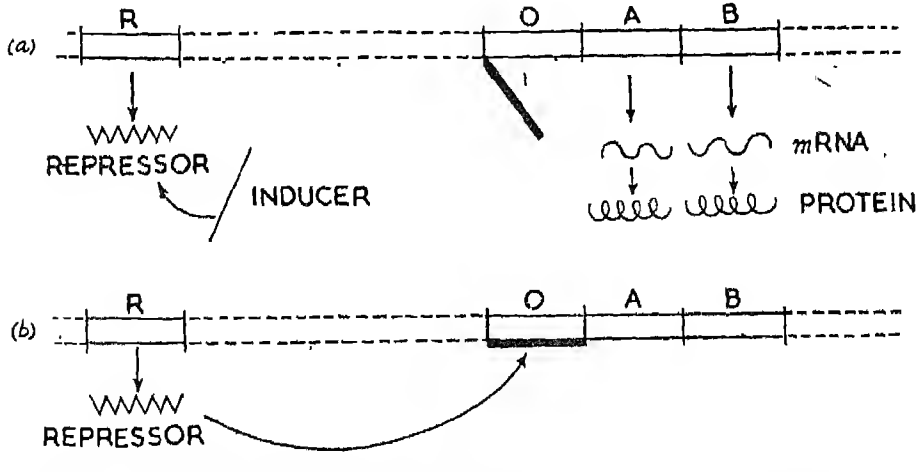


Fig. 13.4 Schematic representation of the Operon concept for the gene regulation and production of protein molecules

coordinated in order to maintain the living state. Our knowledge is still in a very incomplete stage but we are confident that as our understanding of these processes of cells increases, so will our ability to control and modify them. And this precisely is the secret of life we want to unravel.

EXERCISES

1. What are enzymes? Discuss their usefulness in life processes.
2. Discuss the mechanism of action of enzymes.
3. What are the limiting factors in the proper working of enzymes?
4. How enzyme actions are regulated?
5. What is the function of an enzyme dehydrogenase?
6. Name three properties common to all enzymes.

UNIT 2

GENETICS

CHAPTER 14

Physical and Chemical Basis of Heredity

CELLS WERE first seen as small chambers in thin slices of cork and other plant materials by Robert Hooke in 1665. But it was not until 1831 that the nucleus was discovered in plant cells by Robert Brown. Since then, the nucleus has been recognised as the most important organelle of the cell. Now, it is well established that the nucleus is the storehouse for all hereditary information and is the control tower of all metabolic functions of the cell.

A typical cell usually contains only one nucleus, but there are cases (as in fungal mycelia) where more than one nucleus are found in a cell. The nucleus remains suspended in the cytoplasm. In young cells, it occupies a central position but as the cell matures, its nucleus is pushed to one corner by the enlarging vacuole (Fig. 14.1). Nuclei are usually oval or spherical, but they may be elongated (as in muscle fibres), lobed (as in human neutrophil cells), branched (as in the silk spinning cells of insect larvae), or of variable shape (as in leucocytes) (Fig. 14.2). The shape and size of the nucleus determine its surface area which is in contact with the cytoplasm.

It was Strasburger who, in 1873, suggested that nuclei arise only from the pre-existing ones and not *de novo*. The fact that the egg and the sperm nuclei fuse at the time of fertilization was independently established by Hertwig (1875) and Van Beneden (1875). It was against this background that Weismann (1883-1885) proposed his theory of the continuity of germplasm, which states that 'heredity is brought about by the transference from one generation to another of a substance with definite chemical, and above all, molecular constitution'. He called this substance germplasm and equated it with a nuclear substance that is passed on from one generation to another.

It was soon realized that the eggs of a given species are much bigger than the sperms, and this difference is mainly because they possess different amounts of cytoplasm. Their nuclei are of the same size. The same is true of male and female gametes of plants. The genetic contributions of both male and

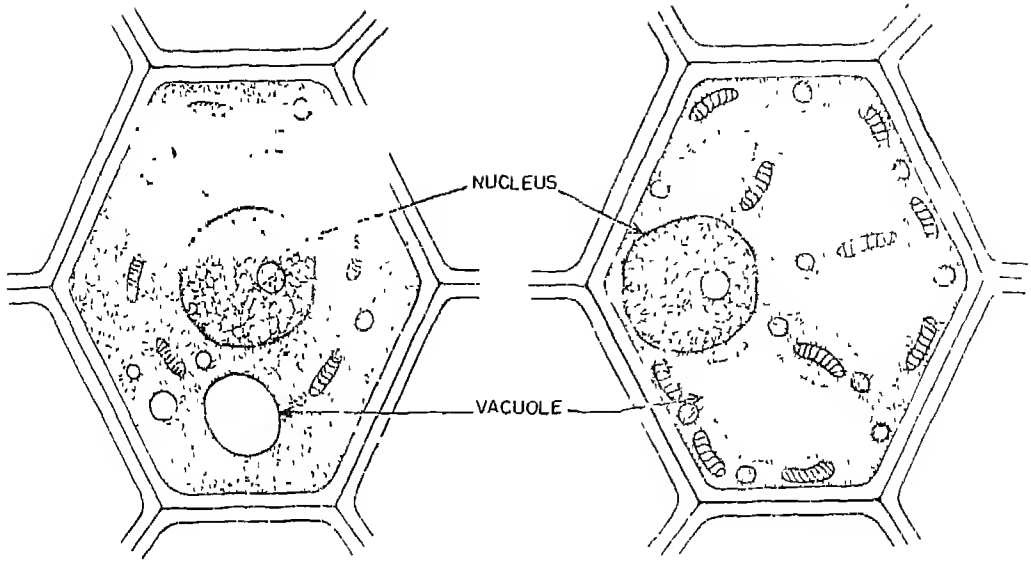


Fig 14.1 A young cell with a nucleus in the centre (*left*) and a mature cell showing the nucleus pushed to one side due to the enlarged vacuole (*right*)

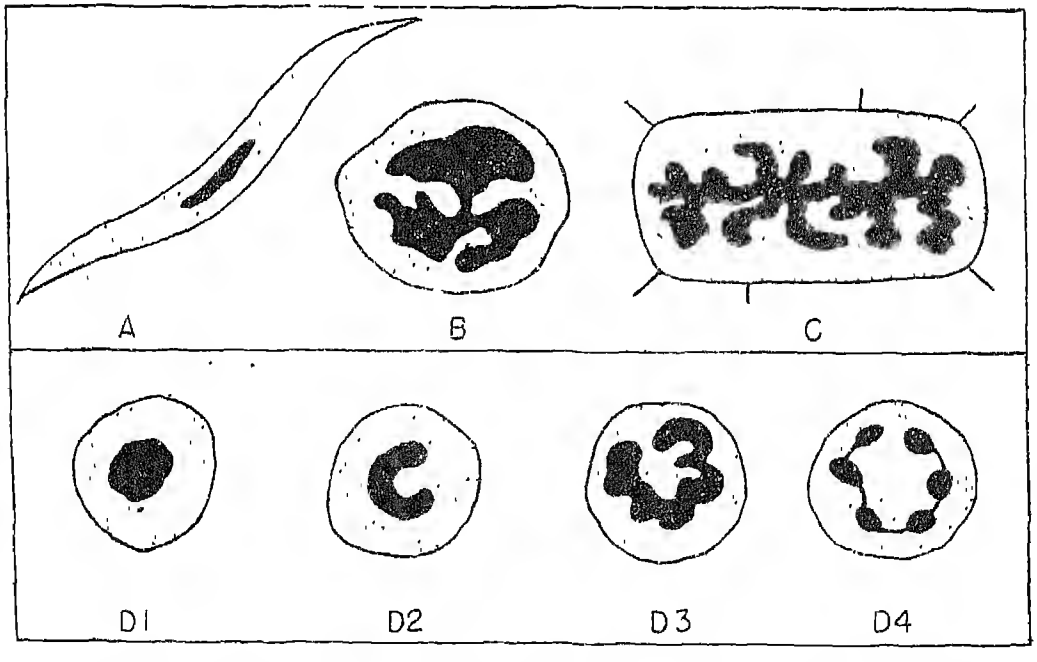


Fig 14.2 Different shapes of the nucleus *A* Elongated in a muscle fibre *B* Lobed in a human neutrophil cell *C* Branched in a silk spinning cell of an insect larva *D* 1 to 4 of variable shapes in leucocytes

female parents are equal, indicating thereby that it is the nucleus and not the cytoplasm that is the carrier of hereditary information The experimental evidence for this fact was provided by Boveri's experiments (1889) on sea urchins. He fragmented the eggs of sea urchins by shaking until they broke into two pieces, one with and the other without a nucleus Even the enucleated part of the egg could be fertilized and made to develop, suggesting thereby that the egg and sperm nuclei are essentially similar in their hereditary contributions and that the sperm nuclei possess all the hereditary information required for growth and development When the enucleated and nucleated egg pieces were fertilized by the same type of sperms, the larvae produced from the enucleated eggs had only paternal characters, whereas those produced from the nucleated eggs had both paternal and maternal characters. The differences between the two types of larvae were due to the absence or presence of the egg nucleus Thus, the nucleus has a definite role in the transmission of characters from one generation to another. Boveri's conclusions gained more support from experiments on amoeba, algae, amphibians and a variety of other organisms The enucleated portion of *Amoeba proteus* gradually becomes inactive and unresponsive to external stimuli and ultimately dies. It is unable to initiate a contractile vacuole but can maintain one if it is included in the enucleated half. On the other hand, the nuclear portion remains responsive to its environment, forms a new contractile vacuole if the original one is removed, grows, ingests food and, in due course, divides to form two daughter cells. Activity of the enucleated portion is restored to normal if it is renucleated These experiments show that a cell cannot survive without

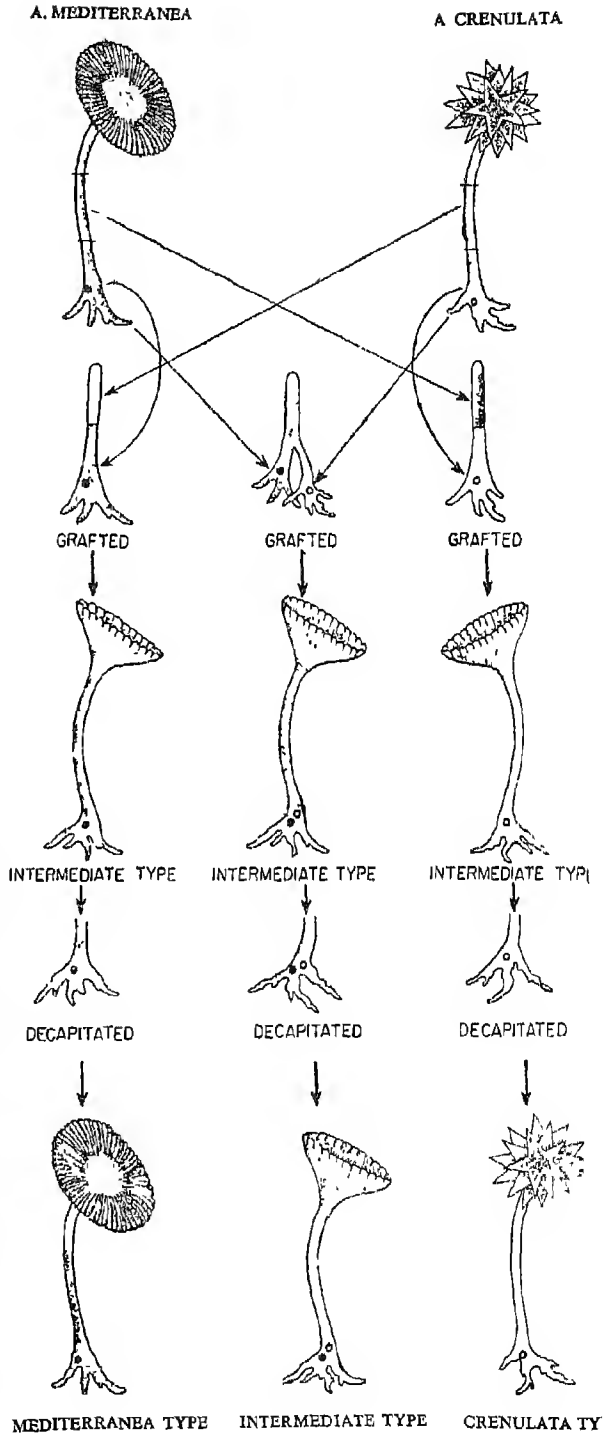


Fig 14 3 Summary of the grafting experiments with *Acetabularia*, to prove that the hereditary characters are determined by the nucleus and not by the cytoplasm

a nucleus and its survival, growth and reproduction are controlled by the nucleus

The role of the nucleus in heredity was firmly established by the grafting experiments of Hammerling (1953) with the unicellular green alga *Acetabularia* (Fig. 14 3). The body of this alga is about six centimetres long and is differentiated into a foot, a stalk and a cap. The cap has a characteristic shape for each species and is easily regenerated if removed. The single nucleus is situated in the rhizoid portion. *A. crenulata* has a cap with about 31 rays, the tips of which are pointed, but *A mediterranea* has about 81 rays with rounded tips. If the cap, stalk or even the nucleated portion of the rhizoid is removed, the remaining portion has the capacity to regenerate into a whole plant. The enucleated part looses the regeneration capacity after a few decapitations, but the nucleated portion always maintains this ability. When the stalk of one species is grafted on to the nucleated rhizoid of the other, an intermediate type of cap is formed. On decapitation, a second cap develops which resembles the cap of the species which provides the nucleus. When the nuclei of both the species are present in the same cytoplasm, an intermediate type of cap develops. Such experiments prove beyond all doubt that the nucleus is the storehouse for, and the control tower of, all hereditary information

An enucleated and unfertilized egg of a sea urchin can be stimulated to divide without fertilization by immersing in a hypertonic solution, but it soon stops to divide and

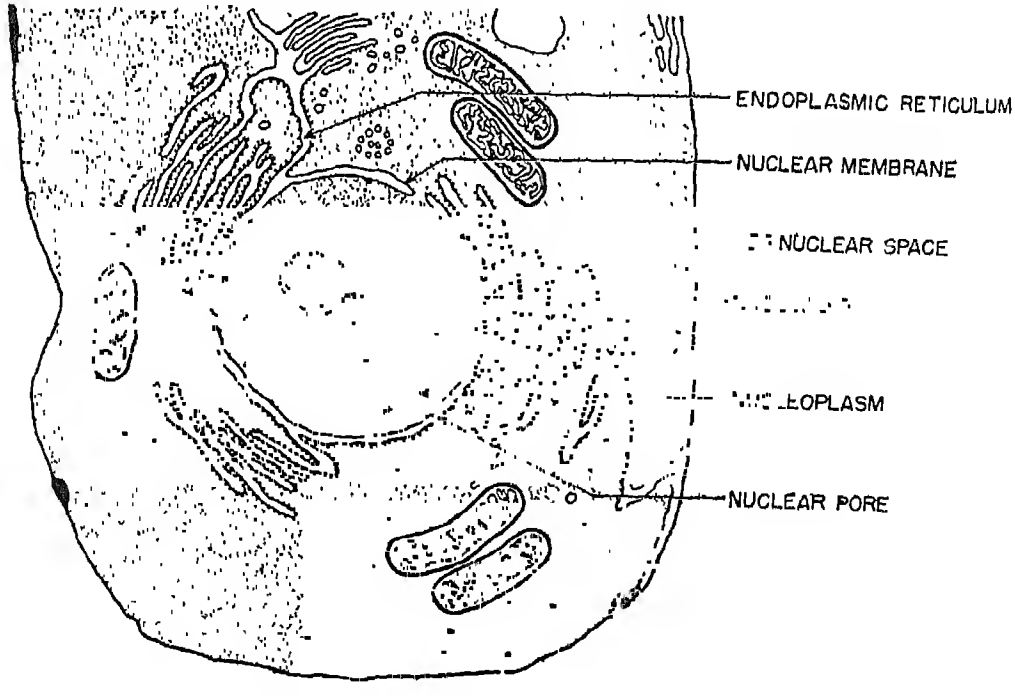


Fig. 14.4 Part of a typical cell showing an interphase nucleus and the continuity of the nuclear envelope with the cytoplasmic membranes.

degenerates Thus, the presence of the nucleus is essential for a continued and proper functioning of a cell The cell cytoplasm is unable to survive and differentiate for long, in the absence of its nucleus At the same time the nucleus without its cytoplasm is unable to survive

The nucleus is surrounded by a nuclear envelope. Election microscopy has revealed that this envelope is a double-membraned structure and has pores which are about 500Å in diameter (Fig. 14.4). The space between the two membranes is known as the perinuclear space or cisterna The outer membrane of the nuclear envelope is at places continuous with cytoplasmic membranes It is through the pores that the cytoplasm communicates with the nucleoplasm (also known as nuclear matrix, nuclear sap or karyolymph) In the living cells, the nucleoplasm appears to be homogenous but on staining with certain dyes, many structures become visible The most obvious amongst these is the knotted threadlike chromatin network which takes up alkaline stains. Sometimes, the chromatin network is not visible and only chromatin granules can be seen instead Each nucleus contains at least one, and often more than one, nucleolus (pl nucleoli) which is usually spherical, dense and rich in proteins and ribonucleic acid. This organelle was first seen by Wagner (1840) and termed nucleolus by Bowman (1840) The nucleolus is always attached to one of the threads of the chromatin network (Fig 15 5) Ribosomes also have been found in the nucleoplasm During the nuclear division, the chromatin network condenses and coils and the long threads gradually become short and thick These darkly staining rodlike structures were called chromosomes by Waldeyer (1888) and were

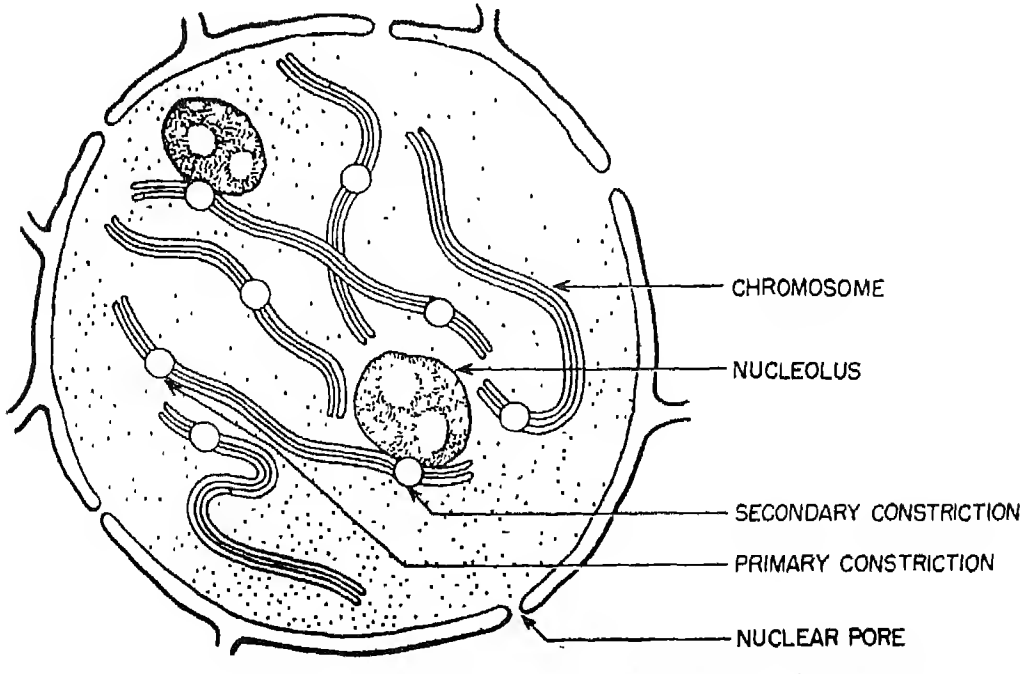


Fig 14.5 A nucleus with its nucleolus attached to specific regions on chromosomes.

first seen by Hofmeister (1848)

Well-defined nuclei and chromosomes are present in the eucaryotic cells. Procaryotes, like bacteria and blue-green algae, have diffuse nuclei (nucleoides) which do not have nuclear membranes, although their nuclear material is aggregated and can be made out clearly from the surrounding cytoplasm in an electron microscope (Fig. 14.6).

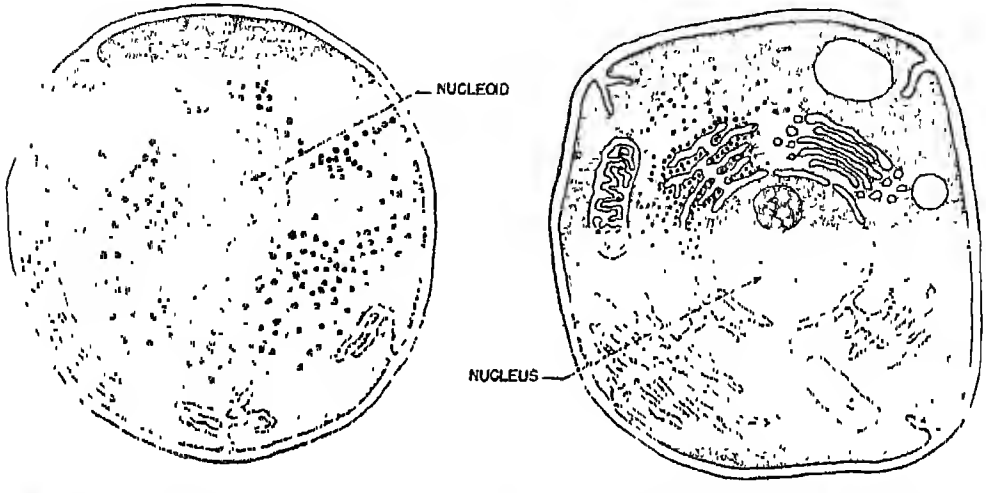


Fig 14.6 Procaryotic (*left*) and Eucaryotic (*right*) cells showing differences in their cellular organization.

The process of fertilization in plants and animals, in which maternal and paternal nuclei fuse, was discovered by Hertwig in 1875. Almost at the same time Van Beneden observed in thread worms that the egg and sperm nuclei contain only two threadlike structures each, while the fertilized egg contains four such structures. The nuclear division of the somatic cells was termed *mitosis* or equational division by Flemming in 1882, who had also observed that just before the division of cells, each chromosome divides longitudinally into two half chromosomes which separate and go to the two daughter nuclei. Thus, the parental as well as the daughter cells possess the same number of chromosomes. The chromosomes maintain their individuality even during the stages when they are not apparently visible.

Gametes are produced as a result of two successive divisions of a somatic nucleus and as a result of this process each of the daughter cells possesses half the number of chromosomes as compared to the parent cell. Detailed studies of this type of nuclear division were carried out by Winiwarter (1900) in rabbits, and Farmer and Moore (1905) coined the term 'meiosis' for this type of reductional division. It occurred to Sutton as well as to Boveri in 1901-1903 that there is a remarkable similarity of behaviour between the characters during inheritance and the chromosomes during reproduction. Maternal and paternal characters blend in the progeny and later on seggregate during the formation of gametes Similarly, the chromosomes from two parents come to lie in the same zygote as a result of the fusion of gametes and again separate as a result of meiosis at the time of the formation of gametes (Fig. 14.7). This led Sutton and Boveri

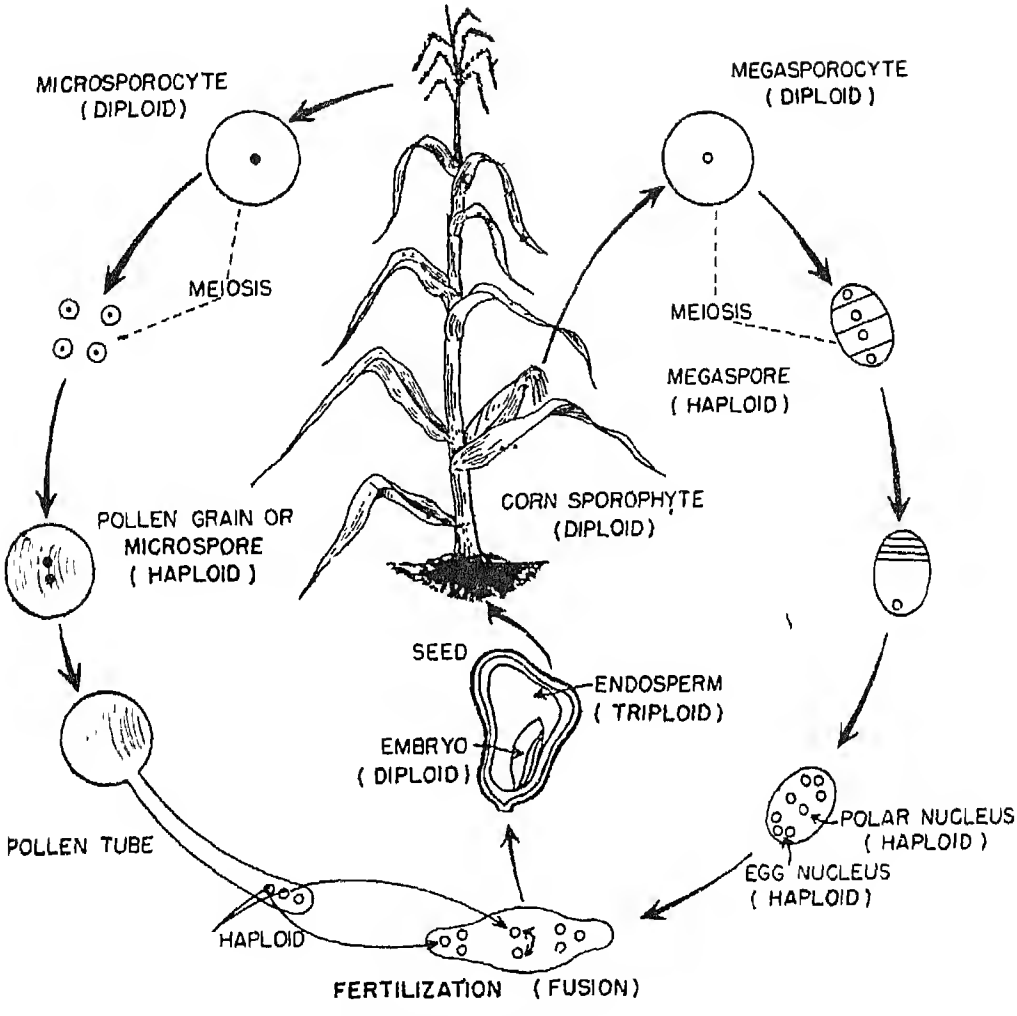


Fig 14 7 Life cycle of a corn plant indicating the stages when meiosis and fusion occur, resulting in the alternation of haploid and diploid phases.

to propose that chromosomes are bearers of hereditary factors which determine the characters of individuals.

Since the nuclei and chromosomes are concerned with the inheritance of characters, it is important to know about their chemical constituents so that the molecular basis of heredity can be understood. The chemistry of nuclei can be studied easily because they can be separated from other cell components by simple chemical and physical techniques. Some staining reactions can also be used to determine the kind and distribution of chemicals that are present in the nucleus and its various organelles. Some of the chemical constituents of the nucleus can be determined by ultra-violet or fluorescent microscopy. Such studies in the last century, have shown that nuclei contain the following

components:

1. Deoxyribonucleic acid (DNA).
2. Ribonucleic acid (RNA)
3. Lipids.
4. Basic proteins (either histones or protamines).
5. Complex proteins, including enzymes.
6. Some phosphorus containing organic components
7. Inorganic components like salts.

Of these, nucleic acids are the unique constituents of the nucleus and are not found in appreciable amounts in the cytoplasm. Nucleic acids were discovered in 1869 by a Swiss biochemist, Friedrich Miescher. He isolated the nuclei of pus cells and, on

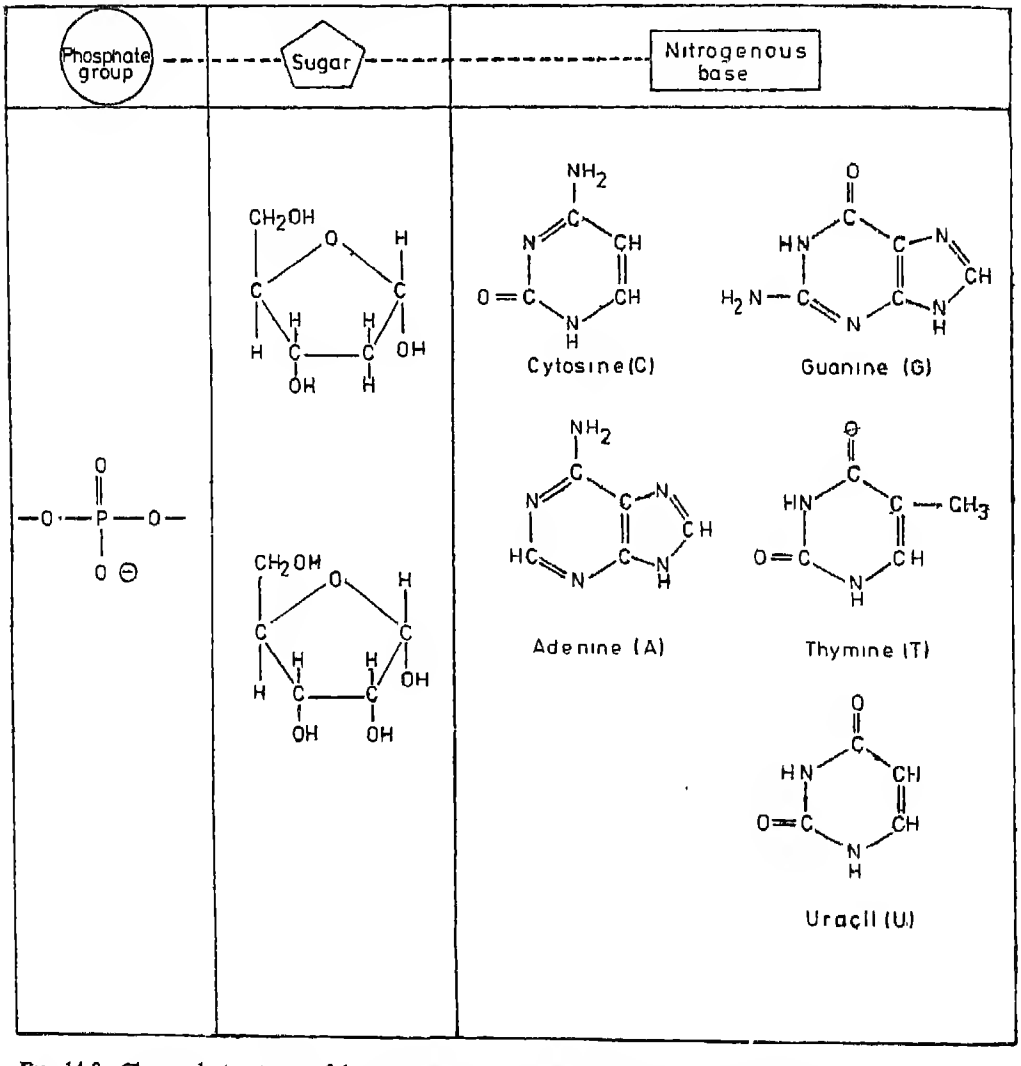


Fig 14.8 Chemical structures of bases, sugars and phosphates that are the building blocks of nucleic acids.

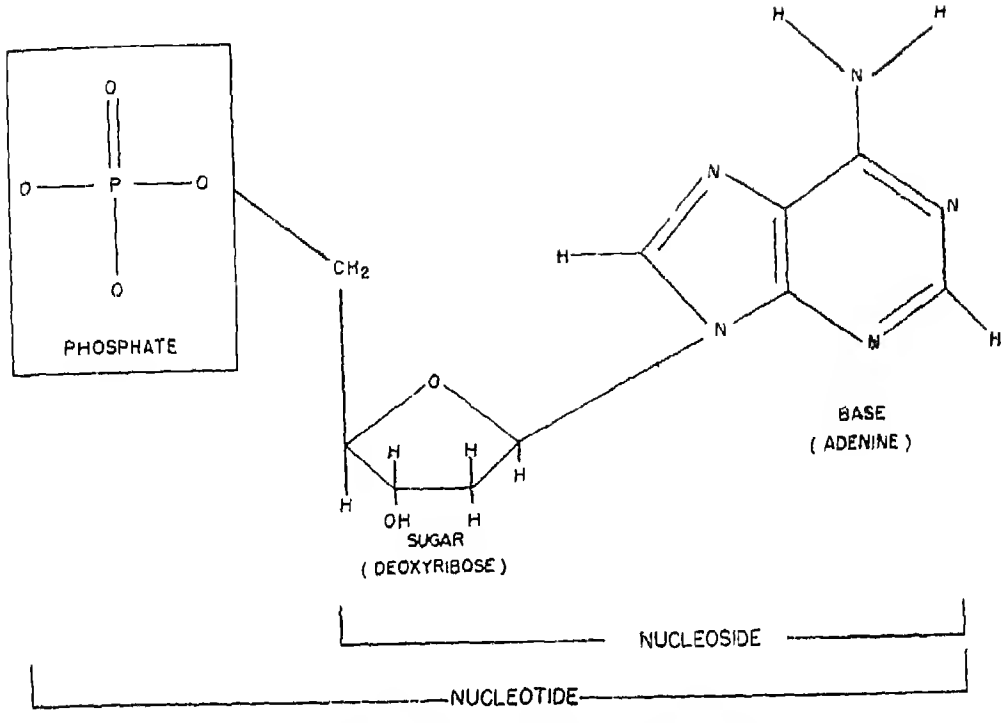


Fig. 14.9 Chemical structure of a nucleotide and a nucleoside.

chemical analysis, found that they contained a chemical the properties of which were unlike those of the then known organic compounds, like carbohydrates, proteins and lipids. Since the new compound was isolated from nuclei, Miescher called it nuclein. Later on, it was called nucleic acid due to its acidic properties. By 1940, it was clear that nucleic acids are of two types: deoxyribose and ribose, depending on the type of sugar they have. Ribosenucleic (or simply ribonucleic) acid is found in the nucleoplasm as well as in the cytoplasm. Nucleic acids are macromolecules and polymers of nucleotides. Each nucleotide consists of five carbon sugars, phosphate and a purine or pyrimidine base (Fig. 14.8). Purines and pyrimidines are nitrogen-containing organic compounds. Nucleic acid, polymers or polynucleotides, can be broken up into its various components by hydrolysis. Complete hydrolysis yields purine and pyrimidine bases, sugar and phosphoric acid. Partial hydrolysis yields smaller polynucleotides, nucleotides and nucleosides (Fig. 14.9) A nucleoside contains a nitrogenous base attached to a pentose sugar molecule. Nucleotides are phosphoric esters of nucleosides. The linkage between adjacent nucleotides is of ester type in which the 5′ and 3′ hydroxyls of two adjacent sugars form a double ester with phosphoric acid (Fig. 14.10). Such an end-to-end joining of nucleotides results in a polynucleotide.

A nucleotide of DNA is composed of a deoxyribose sugar, phosphate and one of the following four bases: adenine, guanine cytosine, and thymine. A nucleotide of RNA, on the other hand, consists of a ribose sugar,

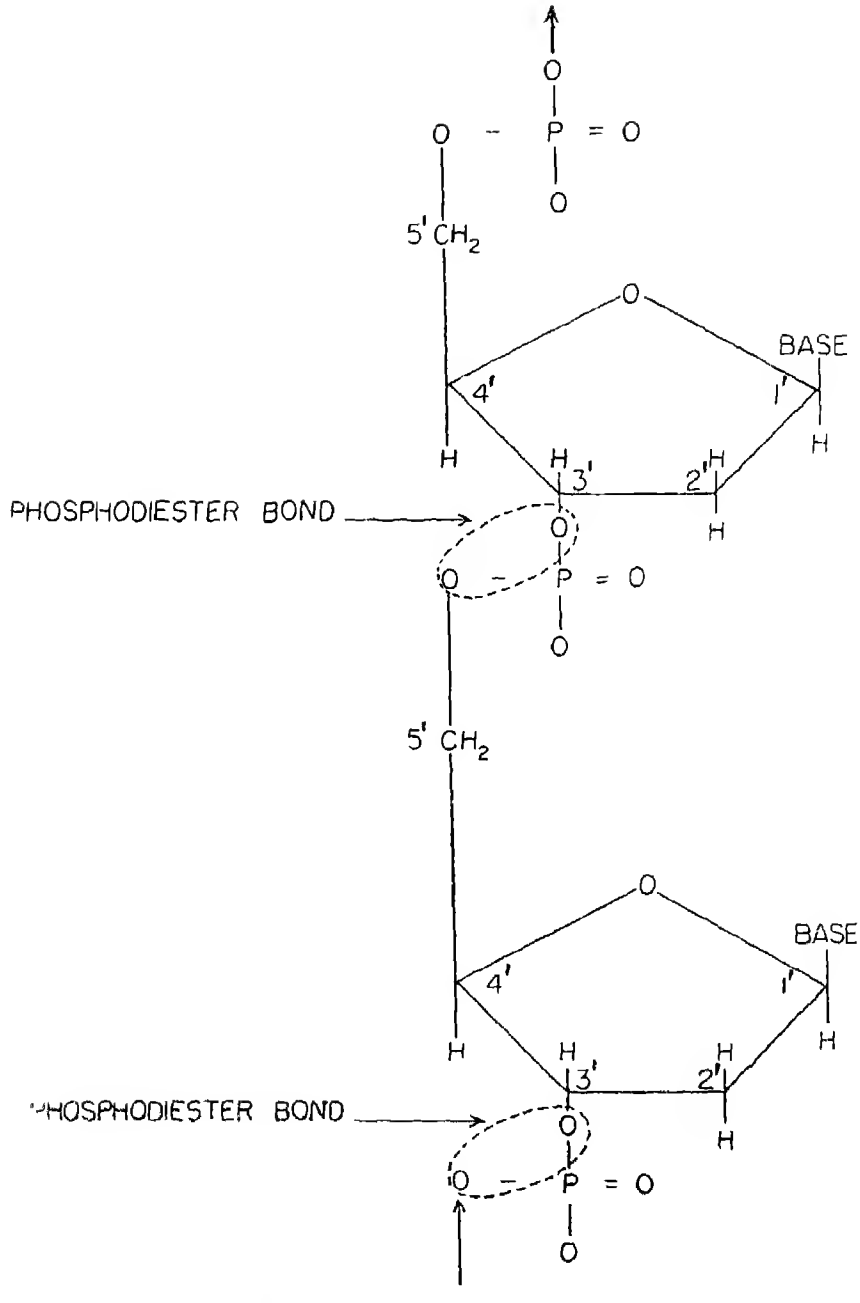


Fig. 14.10 Phosphodiester bonds that link nucleotides to form a polynucleotide

phosphate and one of the following four bases: adenine, guanine, cytosine, and uracil. Of the five bases of nucleic acids, adenine and guanine are purines whereas thymine, cytosine and uracils are pyrimidines. Thus, besides its sugar, RNA differs from DNA in having uracil instead of thymine.

In 1950, Erwin Chargaff estimated the relative amounts of various bases in DNAs that were extracted from different sources (Table 14.1) He found that regardless of the source of DNA, the molar amount of adenine always equalled that of thymine. Because of this relationship, the concentration of purines in the tissues was always the same as that of pyrimidines. But DNAs extracted from different organisms showed variations in absolute quantities of purines and pyrimidines, which is the characteristic of a given species. When DNA from various tissues and organs of the same species was analysed, its composition was found to be the same.

TABLE 14.1

Base Composition of DNA Extracted from Various Organisms

Source of DNA	*Purines*		*Pyrimidines*		*Per cent* $(G+C)$	*Per cent* $(A+T)$
	Adenine	*Guanine*	*Cytosine*	*Thymine*		
Yeast	31.3	18.7	17.1	32.9	35.8	64.2
Human sperm	31.0	19.1	18.4	31.5	37.5	62.5
Salmon sperm	29.7	20.8	20.4	29.1	41.2	58.8
Wheat	27.3	22.7	22.8	27.1	45.5	54.4
Bacterium *E coli*	26.0	24.9	25.2	23.9	50.1	49.9
Tuberculosis bacterium	15.1	34.9	35.4	14.6	70.3	29.7

X-ray diffraction patterns of DNAs isolated from various organisms were found to be essentially the same by Wilkins, Franklin and Astbury. It also suggested that the DNA molecule is helical rather than linear. In 1953, Watson and Crick proposed a model of DNA (Fig. 14.11) which takes into account its chemical, physical and biological properties. They suggested that each DNA molecule consists of two polynucleotide chains which are helically coiled around a common axis. The two chains are held together in position due to covalent hydrogen bonds between their paired bases. Adenine of one polynucleotide chain pairs with thymine of the other and guanine with cytosine. If the sequence or order of bases of one polynucleotide chain is known, that of the other can be easily determined. Thus, the two polynucleotides of a DNA are complementary to each other. Alternating sugar and phosphate molecules form the backbone of each polynucleotide chain. Watson, Crick and Wilkins were awarded the Nobel Prize for Medicine in 1962 for unravelling the structure of DNA. Unlike DNA, RNA is generally single-stranded. It is a polynucleotide of ribonucleotides.

Much of the DNA is localized in chromosomes of the nuclei. In fact, chromosomes are composed of about 40% DNA, 50% histones and other basic proteins, 1.5% RNA and 8.5% acidic proteins. In each chromosome, a single long DNA double helix is folded and coiled and is associated with histones to form a thick rodlike structure.

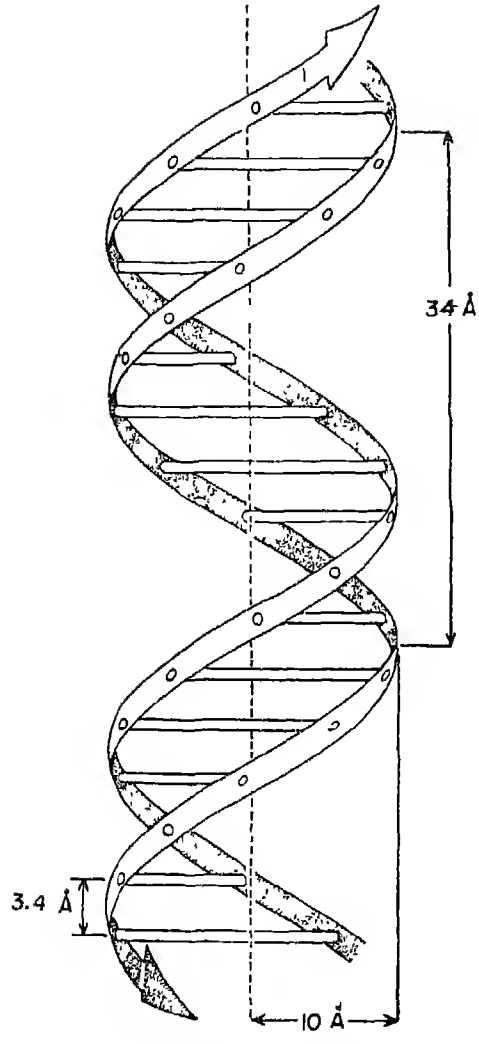


Fig. 14.11 The double helix model of DNA. The width (diameter) of the helix is 10A° and the turn of the helix is completed in 3.4A° which accommodates ten stacks of base pairs.

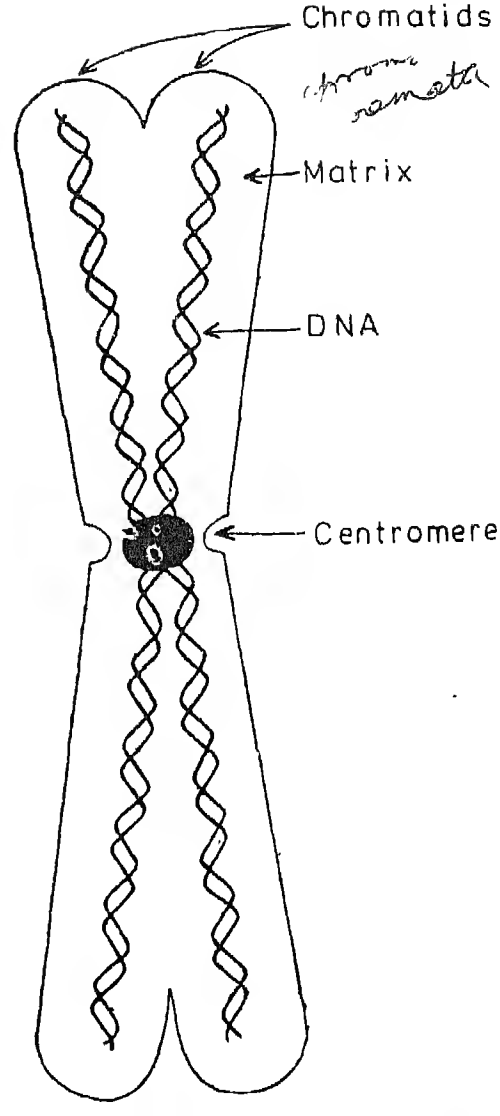


Fig. 14.12 Diagrammatic representation of different parts of a chromosome.

Depending on this coiling and condensation, chromosomes appear short and thick or long and thin. During the interphase, they are so long that they cannot be seen with an optical microscope. Chromosomes are the shortest during the metaphase of the cell division. Under the microscope, each chromosome appears to have a homogeneous matrix in which there are two coiled threadlike structures which are called *chromonemata.* The two chromonemata of the chromosome are joined together at the centromere, which

is also known as the primary constriction (Fig 14.12). Some chromosomes have secondary constrictions (Fig. 14.5) which are also known as nucleolar organisers, because at this place the nucleoli appear and disappear during the cell division.

Although it was known just after the turn of the present century that chromosomes are the bearers of hereditary characters, the role of its DNA in heredity was firmly established as a result of a series of experiments which had their beginning in 1920. S. F. Griffith, a British doctor, conducted some experiments with *Diplococcus pneumoniae*

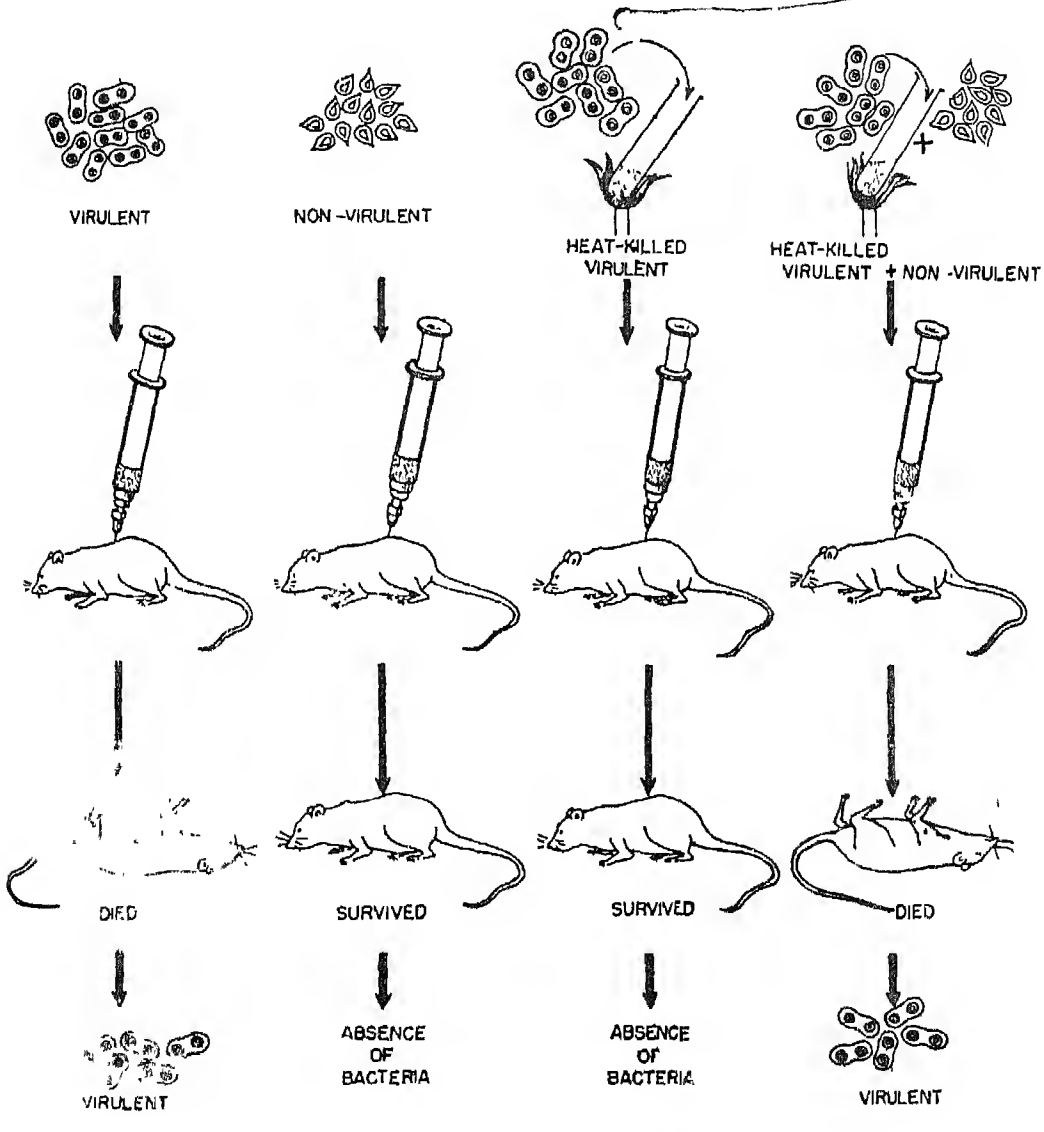


Fig 14 13 **Diagrammatic summary of Griffith's experiments on transformation in *Diplococcus*.**

(commonly known as pneumococcus), a bacterium that causes pneumonia There are two main kinds of pneumococcus bacteria, smooth (S) and rough (R) The S-type cells have a capsule around each pair, are virulent and cause pneumonia. On the other hand, the R-type cells have no capsule and are avirulent or harmless. The S-type cells cause pneumonia and subsequent death when they are injected into mice. The R-type cells fail to cause pneumonia in the mice that are injected with it. Griffith killed the virulent type (S) cells by heat and injected the dead cells into mice The mice did not die or suffer from pneumonia In another experiment, he injected into the body of mice a mixture of living cells of the non-virulent R-type and the heat killed virulent S-type cells. These mice suffered from pneumonia and ultimately died (Fig 14.13). Autopsies showed that death was caused by the living cells of the virulent (S) strain. Griffith concluded that it was the living but non-virulent (R) bacteria that had been transformed into the virulent and capsulated type (S), i e, the dead cells of the S-type bacteria had transmitted their virulence to the living R-type cells A few years later, other scientists showed that the S-type heat-killed cells can transmit their virulence to the R-type living cells when they are placed together in a culture medium, proving thereby that the mice cells or tissues have no role to play in the transformation of one bacterial type into another. Another group of scientists went a step further They prepared an extract of heat-killed S-type cells by growing and disrupting them Even this extract was capable of converting the R-type cells into the S-type in culture media. This experiment proved that some component of the cell, rather than the whole cell, is responsible for transformation

O T Avery, C Macleod and M McCarty (1944) hunted for the transforming compound in the extract of pneumococci and provided the experimental evidence for the fact that the factor responsible for the transformation of the non-virulent R-type bacteria was DNA. They took advantage of the fact that the enzyme deoxyribonuclease digests and decomposes DNA These three scientists separated the extract of the S-type virulent strain into protein, DNA and carbohydrate fractions Each of these fractions was then mixed with the living R-type cells in a culture medium and left undisturbed for a short time. They found that when the protein or carbohydrate extracts from the virulent strain were used, only the S-type cells were produced. But a mixture of the R-type cells and the DNA from the virulent cells produced both the R- and the R-type cells When the DNA extract was treated with deoxyribonuclease (an enzyme which digests DNA) before mixing with the non-virulent type, only the R-type cells were produced The results demonstrated beyond all doubt that the DNA of virulent pneumococci is capable of transforming the R-type cells into those of the S-type Thus, the transformation experiment indicated that DNA is the genetic material and that it is not destroyed by killing the cells by heat treatment Conclusive evidence that DNA and only DNA is the genetic material has since been obtained in a number of direct and indirect ways, but perhaps most convincingly by an experiment with a bacteriophage (a virus that infects a bacterium)

Bacteriophages reproduce within the bacterial cells and eventually may destroy the very cell they have inhabited. The electron microscopy has revealed that the inner portion of a bacteriophage is injected into the bacterial cell and the protein coat remains outside. The protein coat can be detached from the infected bacterium by vigorous

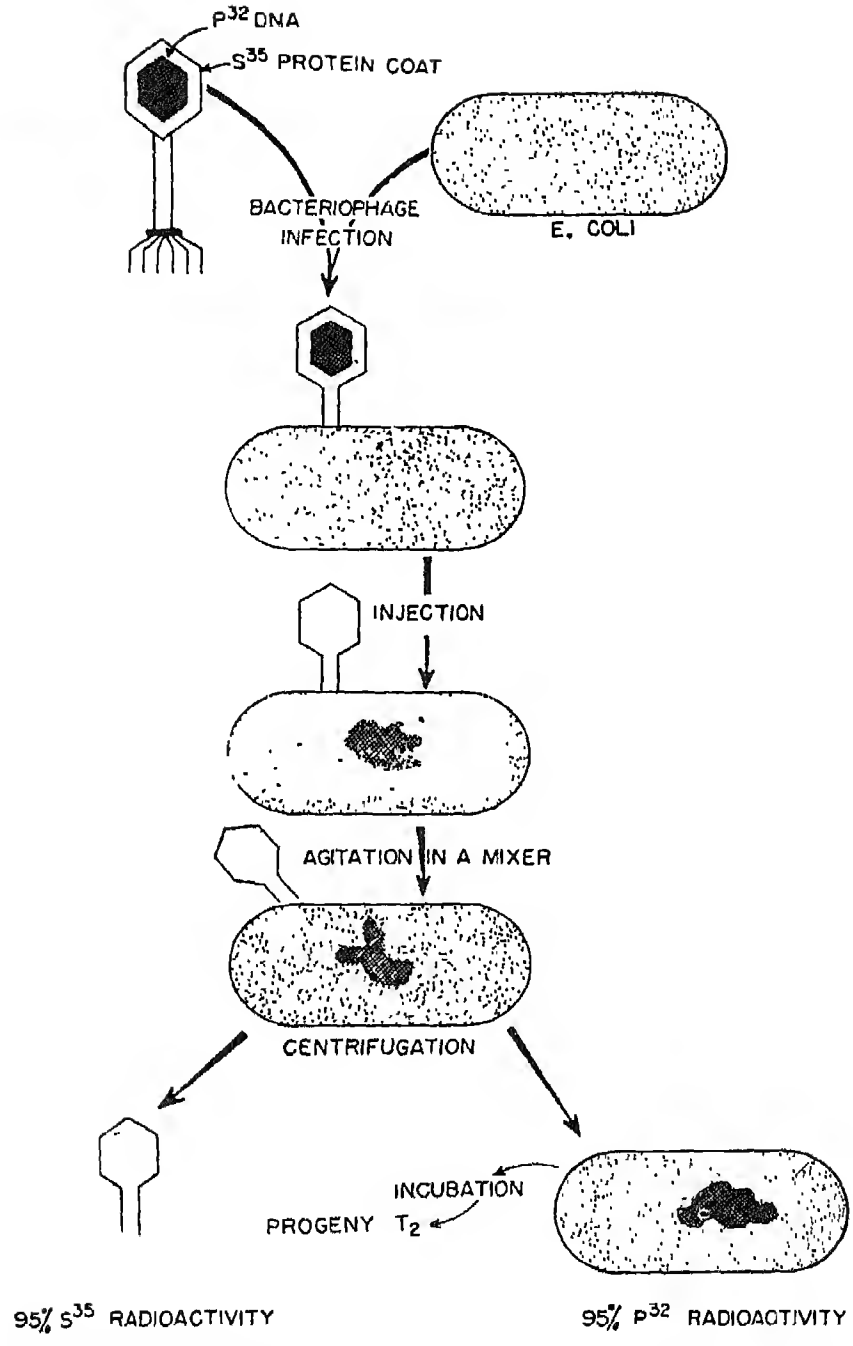


Fig. 14 14 Experimental design to prove that genetic information in the bacteriophage T_2 is transferred from one generation to another by its DNA.

shaking and subsequent centrifugation. Protein coats being lighter stay in the supernatant and the infected bacteria settle down in a centrifuge tube. The tadpole-shaped bacteriophage T_2 which infects the bacterium *Escherichia coli* has a DNA core and a protein coat. Hershey and Chase (1952) set out to find whether during infection only DNA is injected or also some of the protein enters the bacterium. They grew *E. coli* in a medium containing S^{35}, a radioactive isotope of sulphur. The bacteria incorporated the hot (radioactive) sulphur into the sulphur-containing amino acids (cysteine and methionine) which were used up as building blocks for proteins. The hot bacteria were then infected with T_2 phages. Since the phages utilize the proteins of bacteria for the formation of phage proteins, they became labelled with S^{35}. Such hot phages were used to infect cold bacteria. After infection, the protein coats and bacterial cells were separated and their radioactivity was measured. Hershey and Chase found that almost all of the radioactivity was associated with protein coats in the supernatant and almost none with the bacterial cell fraction. This indicated that no protein material of the phage had entered the bacterial cell in the course of the infection process. The experiment was repeated by using P^{32}, a radioactive isotope of phosphorous which gets incorporated into DNA rather than into the proteins. Hershey and Chase found in this experiment that almost all the radioactivity was associated with bacteria rather than with the protein coat fraction (Fig. 14.14). These experiments proved that all the phage DNA and only the DNA entered the bacterium. The more important finding was that the bacteria which contained the injected DNA, but were separated from the phage coat protein, were capable of producing a fresh crop of normal bacteriophages. Since the only link between the infecting and the progeny bacteriophages is the injected DNA, it is the DNA and not the protein which is the hereditary material of T_2. We now know it for certain that in all organisms DNA is the hereditary material and it is the DNA part of the nucleus and chromosomes which is responsible for heredity. In those organisms in which there is no DNA, RNA takes over the hereditary function of DNA, as in the tobacco mosaic virus. Otherwise, it helps in amplifying and transmitting the genetic information that is contained in the DNA.

EXERCISES

1. Give at least two arguments to suggest that the nucleus contains the hereditary information.
2. The shape of the cap of *Acetabularia* is determined by the type of the nucleus or the type of the cytoplasm that it has.
3. What is the function of nuclear pores?

4. What is the major difference between a procaryotic and an eucaryotic cell?
5. What is meant by the parallelism of behaviour between chromosomes and characters? What do you conclude from this?
6. What are the major chemical constituents of chromosomes and which one of them carries the genetic information?
7. What is the relationship between the following: bases, sugars, phosphate, nucleoside and nucleotide?
8. If the base sequence of one strand of DNA is CAT TAG CAT CAT GAC, what will be the base sequence (a) of complementary DNA strand, and (b) of its complementary RNA strand?
9. Which experiments led to the conclusion that DNA is the chemical basis of heredity?
10. Can RNA function as the hereditary material?
11. What is the chemical that brings about transformation?
12. Do bacteriophages inject their nucleic acid core or their protein coat into the bacteria that they infect? How can you prove this?

CHAPTER 15

Functions of Nucleic Acids

SELF-DUPLICATION is the most important feature of living organisms. Cell division plays an important role in it. This process is very precise and maintains similarity between the parent and daughter cells. As the characters of a cell and an organism are controlled by their nuclei, chromosomes and DNA, it is essential that during cell division the nucleus and its chromosomes as well as its DNA make their copies with great precision and exactitude. Besides making its precise copy, the hereditary material should be able to direct and control the various metabolic functions of the cell and ultimately of the organism as a whole. Only this can ensure the continuity of a species and the maintenance of its characters from generation to generation. DNA carries out both these functions by replicating and by expressing the genetic information contained in its base sequence.

Replication of DNA

While suggesting their model of DNA structure, Watson and Crick postulated a mechanism for its replication. They suggested that at the time of replication two complementary strands of DNA unwind and separate from one end in a zipperlike fashion. During this process, the weak covalent hydrogen bonds between the bases are broken. The separated single strands act as molds or templates for the synthesis of new strands (Fig. 15.1). Thus, two daughter DNA double helices are formed which are identical to each other and to the parent molecule. As a result of such replication, the DNA helices will be half old and half new. In other words, during replication, half of the DNA molecule is conserved and its complementary half is freshly synthesized according to the sequence of bases contained in the conserved half. This mode of replication, therefore, has been called semi-conservative and has been experimentally proved to be true.

The semi-conservative mode of replication was proved by Messelson and Stahl of California Institute of Technology in 1958. They grew *Escherichia coli* for many generations in a culture medium in which the nitrogen source contained only the heavy isotope N^{15}. This resulted in the labelling of all the bacterial DNA with N^{15}. Then, the nitrogen source was changed to N^{14}, which is the normal light isotope of nitrogen, and the bacteria allowed to grow. Cell

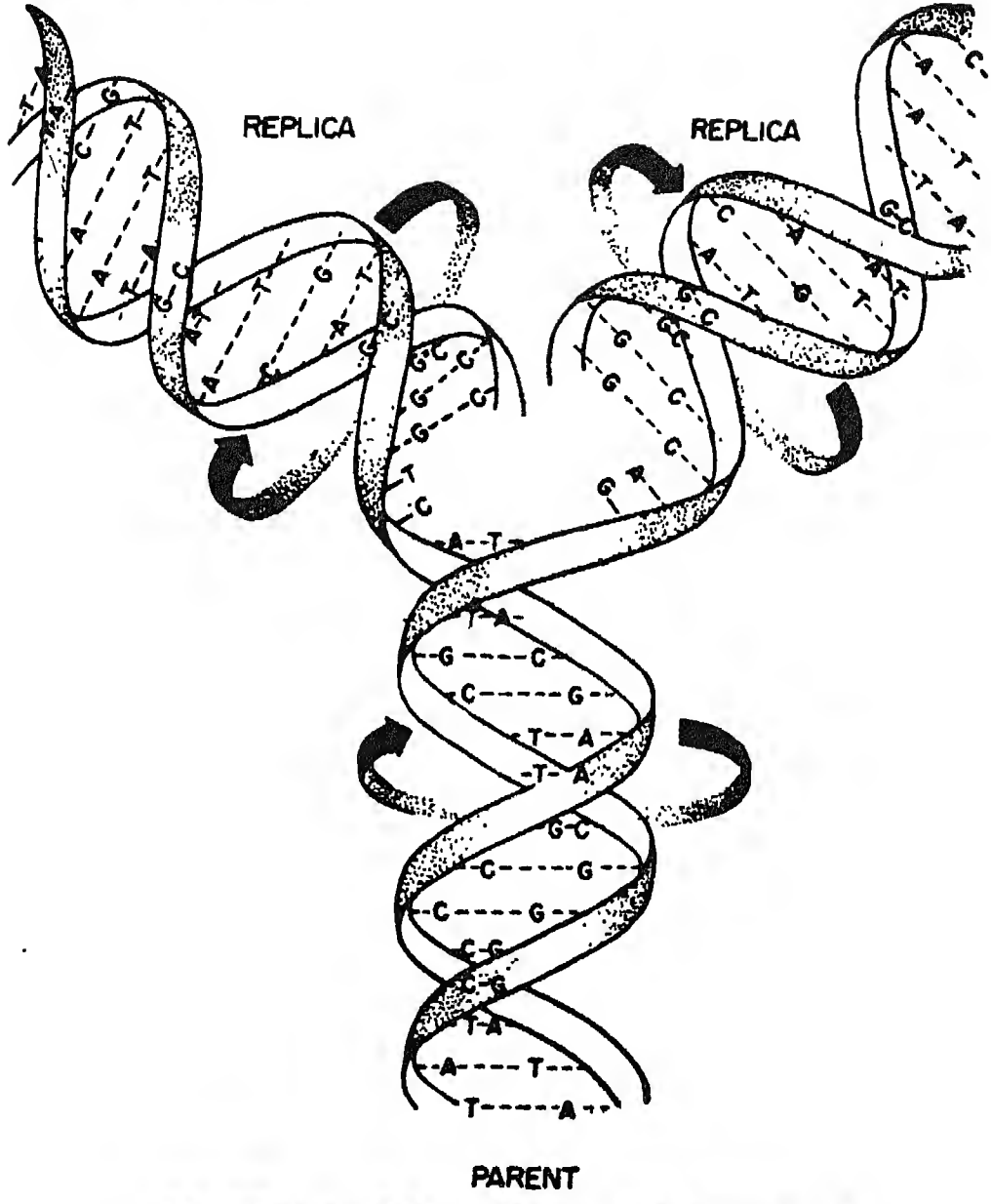


Fig. 15.1 Semi-conservative mode of DNA replication, as proposed by Watson and Crick

samples were removed at regular intervals,, DNA extracted from them and analysed for its density. This experiment showed that when the double stranded DNA containing N^{15} in both of its stands heavy was allowed to replicate once in the absence of N^{15} (i.e., in the presence of N^{14}), one stand of each daughter DNA moleçule remained heavy,

whereas the other one was light During second replication, the light and heavy strands separated and served as templates for the synthesis of light strands Out of the two daughter DNA molecules, one remained half heavy and half light, whereas the other one was completely light (Fig. 15.2). This biochemical evidence was supported, a few years later, by direct cytological observations of duplicating DNA of *E coli*

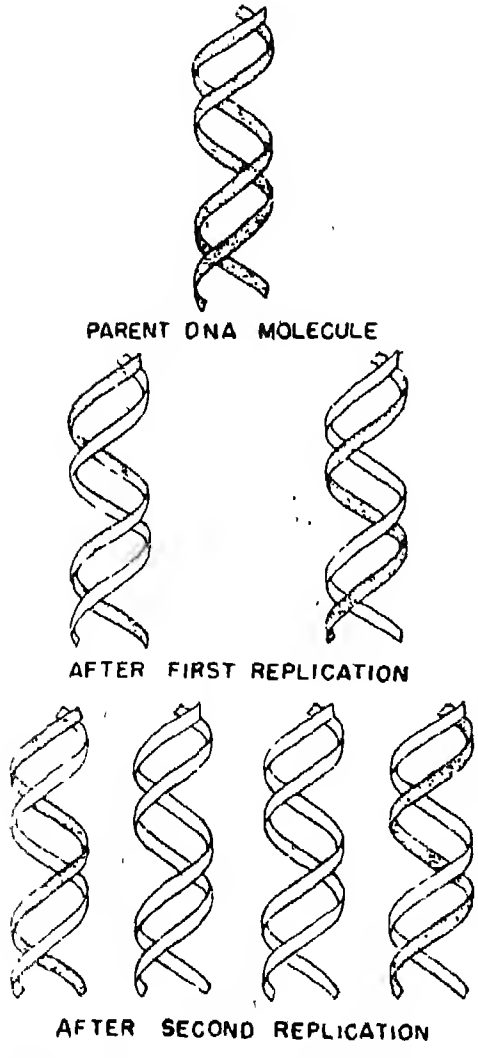


Fig 15 2 Distribution of density as a result of semi-conservative replication Radioactive strands are shaded whereas light ones are non-shaded.

Since DNA is contained in chromosomes, the latter also should show semi-conservative replication. In fact, semi-conservative duplication of chromosomes was demonstrated by Taylor in 1957, but in the absence of any knowledge of the mode of organization of DNA in chromosomes, its significance was not realized until later. Taylor supplied thymine (one of the DNA bases), labelled with H^3 (radioactive isotope of hydrogen), to the dividing root tip cells of broad bean The radioactive base got incorporated in the newly synthesized chromosome parts. He then replaced radioactive thymine with non-radioactive thymine and allowed the cells to grow and divide. On cytological analysis he found that initially the whole chromosome got labelled. But during the second division, which occurred in the presence of non-radioactive thymine, only half the chromosomes retained radioactivity. The pattern of distribution of radioactivity in dividing chromosomes of broad bean was very similar to the pattern of distribution of density in replicating DNA of *E. coli* If we assume that each chromosome consists of a double-stranded DNA, the semi-conservative replication of chromosomes can be visualized as depicted in Fig. 15.3.

The molecular mechanism of DNA replication is more or less completely understood and can be achieved even in test-tubes. A single-stranded DNA acts as a template for the synthesis of its complementary strand. Since guanine must always pair with cytosine and thymine with adenine, the sequence

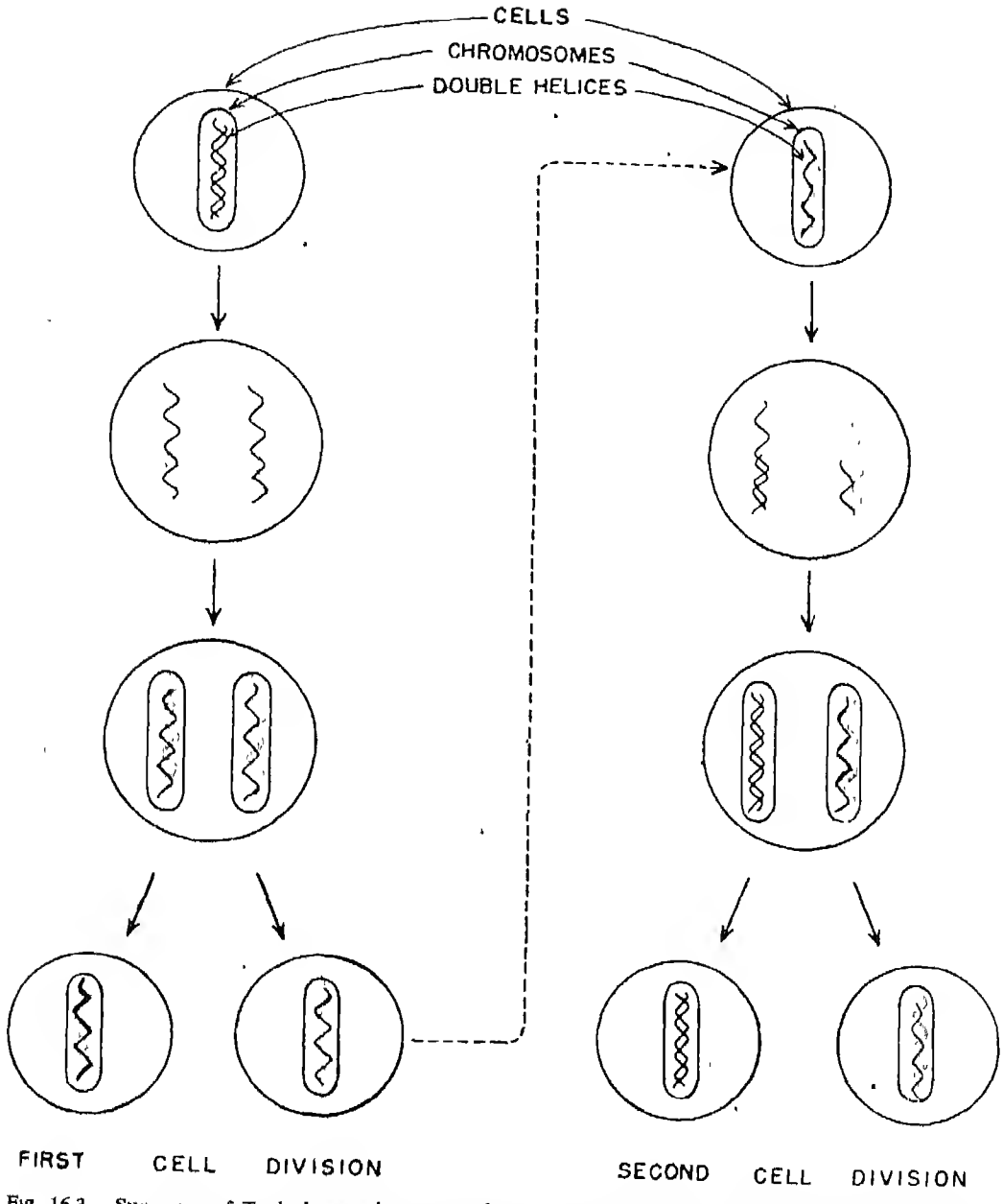


Fig. 16.3 Summary of Taylor's experiments on chromosome duplication. The first cell division occurred in the presence of radioactive thymine, thereby making all the chromosomes radioactive. Radioactive thymine was withdrawn during the second cell division and consequently only half of the chromosomes retained radioactivity. DNA strands shown black are non-radioactive and coloured ones are radioactive.

of bases in the template strand determines the sequence of bases that are to be aligned in the newly synthesized strand. Once the nucleotides are arranged in the proper sequence, they are joined together by the enzyme DNA polymerase. It has also been found that DNA is synthesized in small pieces at a time and these bits are joined together by the enzyme ligase to form long DNA strands. In the living cells, DNA replication occurs with great precision and incredible speed.

Transmission of Genetic Information

DNA is the storehouse for all biological information. It contains instructions for the synthesis of all other molecules of the cell. In most cells, it remains confined within the nucleus. But most of the cell activities take place in the cytoplasm. Therefore, three questions arise: (a) In what language is the genetic information written in DNA? (b) How is this information carried from DNA to the cytoplasm? (c) In what form is the information expressed?

All the metabolic reactions of a cell are catalyzed by enzymes which are proteins. Some of the proteins are structural components of cell organelles. Proteins, like nucleic acids, are macromolecules. They are formed by end-to-end joining of a large number of amino acids There are twenty standard amino acids that go to form polypeptide chains of proteins. The sequence of amino acids in a polypeptide (a chain of amino acids joined together is called a polypeptide chain because each amino acid is linked to its neighbouring amino acid by a peptide bond) is determined by the sequence of bases in a DNA segment. A protein may consist of one or more polypeptide chains. Haemoglobin is a protein which consists of four polypeptide chains — two of one kind and the other two of another kind. Lysozyme is made up of only one polypeptide chain.

Protein synthesis takes place in the cytoplasm and various types of RNA molecules are involved in it. This is evident from the fact that the cells which synthesize more protein have more RNA. One type of RNA carries the instructions coded in the DNA of the nucleus to the cytoplasm. This type of RNA is known as messenger RNA (mRNA) because it carries the message from DNA to the site of protein synthesis. One of the strands of a DNA molecule acts as a template for the synthesis of messenger RNA. During the process of RNA synthesis on the DNA template, adenine is aligned in front of thymine of DNA, uracil in front of adenine, cytosine in front of guanine and guanine in front of cytosine Linking together of these bases with the enzyme RNA polymerase results in the synthesis of a RNA molecule which is complementary in base sequence to one of the strands of DNA. RNA moves from the nucleus to the cytoplasm and becomes attached to a ribosome Ribosomes are small granular bodies which are found attached to the endoplasmic reticulum. They are composed of RNA and protein. Ribosomal RNA (rRNA) is synthesized in the nucleus and transported to the cytoplasm where various types of RNA and proteins get organized to form ribosomes Ribosomes can be purified from disrupted cells, free from other cell components and can be seen under an electron microscope. Provided with a message, building blocks of proteins (i.e , amino acids), and a transporting molecule which can carry the amino acids to the site of protein synthesis, ribosomes are able to synthesize proteins. The amino acid transporting molecule is also a type of RNA which has been given the name of transfer RNA (tRNA). Each amino acid is picked up by a specific kind of tRNA

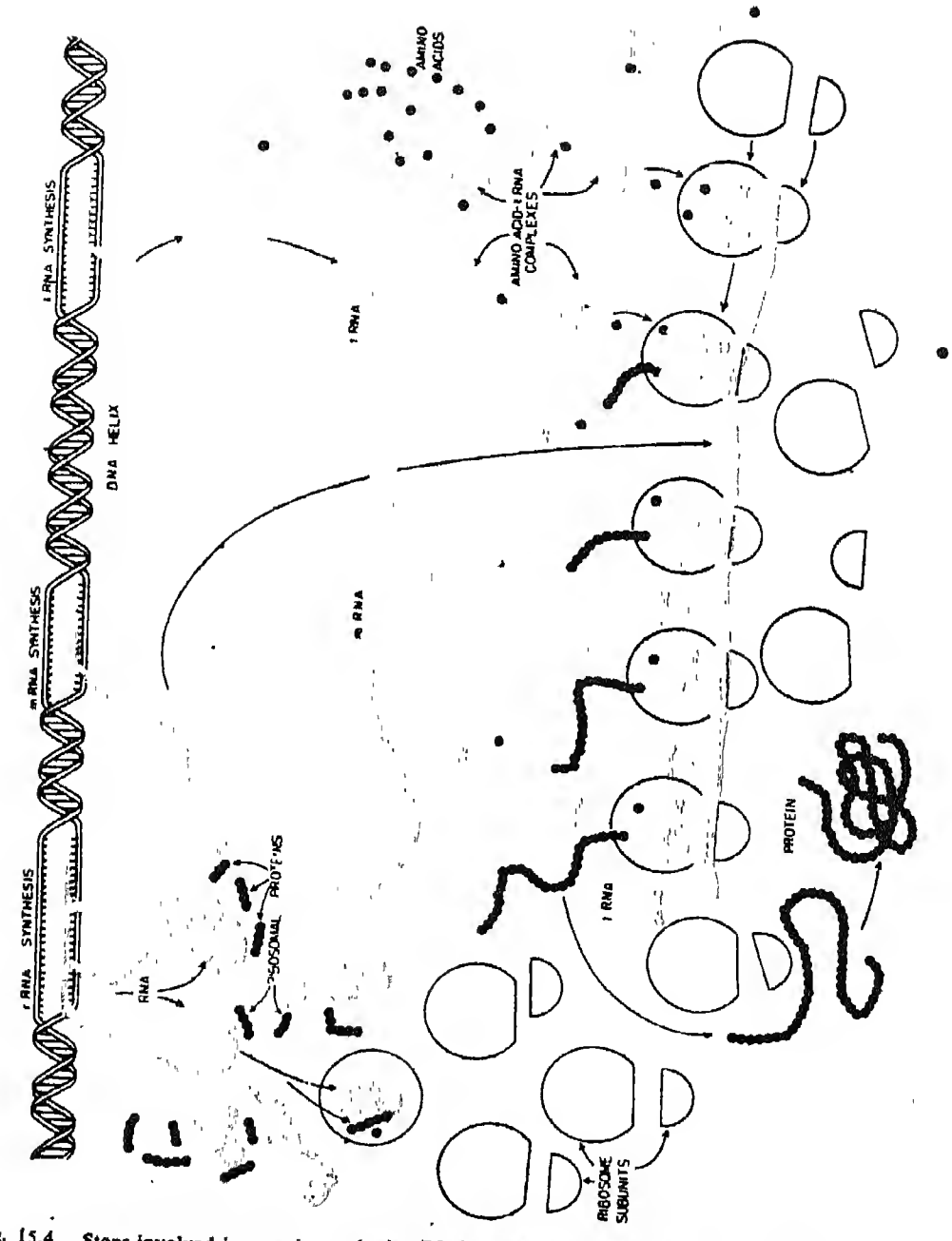


Fig. 15.4 Steps involved in protein synthesis. DNA acts as a template for the synthesis of rRNA, tRNA and mRNA; rRNA and protein combine to form ribosomes which attach themselves to mRNA, tRNA-amino acid complexes align themselves on mRNA-ribosome complexes, two at a time, due to codon-anticodon pairings. The amino acid of one tRNA is linked to the amino acid of the other tRNA, thereby liberating one tRNA. This process continues until a complete polypeptide is synthesized and released.

and the attachment between the amino acid and its tRNA is brought about by an enzyme. tRNAs carry the amino acids to the mRNA-ribosome complex and order them according to the message contained in the mRNA. Later on, these amino acids are hooked together by peptide bonds to form a polypeptide or a protein. The same messenger RNA and ribosome can facilitate the synthesis of many molecules. Thus, DNA makes RNA and RNA makes protein. The process of RNA synthesis on a DNA template is known as *transcription* and the synthesis of protein according to the message contained in mRNA is called *translation*.

$$\text{DNA} \xrightarrow{\text{transcription}} \text{RNA} \xrightarrow{\text{translation}} \text{Protein.}$$

As each ribosome moves along the mRNA, it manufactures a polypeptide chain by translating the base sequence into an amino acid sequence (Fig. 15.4). Many ribosomes can be involved in the translation of a single message, one after the other. A complex of ribosomes attached to a single mRNA can be isolated and has been called a *polysome* or a *polyribosome*.

The information for the sequence of amino acids of a protein is contained in the sequence of bases of the mRNA which in its turn is governed by the nucleotide sequence of the DNA. In other words, a four-letter language of DNA is transcribed into a four-letter language of RNA which is translated into a twenty-letter language of proteins. The language or code of DNA is written by four letters — A, T, G and C. It is now well

TABLE 15.1

The Genetic Code or the mRNA Triplets for Different Amino Acids. Corresponding Bases of DNA are Given in Parenthesis.

Base 1	*Base* 2				*Base* 3
	U(A)	*C(G)*	*A(T)*	*G(C)*	
U (A)	Phe	Ser	Tyr	Cys	U (A)
	Phe	Ser	Tyr	Cys	C (G)
	Leu	Ser	Ochre*	Nonsense*	A (T)
	Leu	Ser	Amber*	Trp	G (C)
C (G)	Leu	Pro	His	Arg	U (A)
	Leu	Pro	His	Arg	C (G)
	Leu	Pro	Gln	Arg	A (T)
	Leu	Pro	Gln	Arg	G (C)
A (T)	Ile	Thr	Asn	Ser	U (A)
	Ile	Thr	Asn	Ser	C (G)
	Ile	Thr	Lys	Arg	A (T)
	Met**	Thr	Lys	Arg	G (C)
G (C)	Val	Ala	Asp	Gly	U (A)
	Val	Ala	Asp	Gly	C (G)
	Val	Ala	Glu	Gly	A (T)
	Val**	Ala	Glu	Gly	G (C)

*Chain termination signals. Ochre and amber also are known as nonsense triplets.
**AUG and GUG stand for chain initiation signals also.

established that each amino acid is coded for by a three-letter word of the nucleic acid. Thus, a sequence of three bases determines as to which amino acid is to be inserted into a polypeptide chain during its synthesis on the mRNA-ribosome complex. A sequence of three bases which stands for an amino acid is known as a code word or a codon. Four bases can form 64 possible codons, but there are only 20 essential amino acids. It follows from this that each amino acid may correspond to more than one codon. As a result of brilliant researches by Nirenberg, Crick, Khorana, and their associates, it has been possible to identify the various code words for the different amino acids. The DNA and RNA code words will be complementary to each other. A glance at Table 15.1 will show that there are two alternative code words for the amino acid phenylalanine — UUU and UUC of RNA and AAA and AAG of DNA. Leucine and arginine have six each, whereas tryptophan and methionine have only one code word each. Other amino acids have either two, three or four codons each. Sixty-one out of sixty-four codons stand for amino acids and the rest three (UAA, UAG and UGA) serve as full-stops. They determine the termination point of a polypeptide chain. The codons AUG and GUG code for methionine and valine, respectively, as well as for start signals. A DNA segment, which is bounded by a start and a stop signal, contains enough information for one complete RNA or polypeptide molecule and is called a *cistron*. Thus, a cistron codes for a polypeptide chain or a RNA molecule. Sometimes, more than one adjacent cistrons code for polypeptides which have related functions or which associate together to form a protein. Such a group of functionally related cistrons has been called a *gene*. Each cistron is transcribed and translated independently of the other. Several mRNA molecules can be transcribed simultaneously from a cistron, one slightly behind the other. Similarly, many polypeptides can be translated from a single mRNA molecule, one after the other, but with the help of different ribosomes.

A triplet code of mRNA is recognised by a particular transfer RNA species. Each species of transfer RNA has two recognition sites. One of them recognises the correct amino acid and the other recognises the mRNA codon due to simple base complementarity. This second site of a tRNA molecule is called an *anticodon*. The amino acids, therefore, are ordered according to the codon sequences of messenger RNA and codon anticodon recognition. The genetic code appears to be the same in all organisms which suggests that it originated quite early during organic evolution.

EXERCISES

1. What are the two major functions of hereditary DNA?
2. How was it proved that DNA replication is semi-conservative?
3. Describe briefly the molecular mechanism of DNA replication.
4. What is the role of messenger RNA?
5. What is the function of ribosomes? How and where are they synthesised?
6. Draw a labelled self-explanatory diagram of the processes of transcription and translation.

7. Fill up the blanks:
   A ..... letter language of DNA is ..... into a ..... letter language of ....., which is translated into a ..... letter language of .....
8. How many bases code for one amino acid? A polypeptide of 1200 amino acids will be coded for by a linear sequence of how many bases of (a) DNA and (b) RNA?
9. Of the 64 possible code triplets, how many stand for amino acids and how many are used as punctuation signals? Are there some which are used as both?
10. The various mRNA code words for the amino acid arginine are: (a) CGU, (b) CGC, (c) CGA, (d) CGG, (e) AGA, and (f) AGG. What will be the corresponding DNA code words or codons?
11. What are the two recognition sites of a tRNA molecule?

CHAPTER 16

Cell Division

SELF-DUPLICATION is the most important feature of a living organism. In the previous chapter, we have seen how DNA (the chemical basis of inheritance) replicates. Each DNA duplication is followed by a separation of the daughter copies and their inclusion in two daughter cells. In most of the plants and animals (exceptions are viruses, bacteria and blue-green algae), DNA is located in the nuclei within the cells. Most of the cellular DNA is formed in the nucleus. Only a small fraction is present in the organelles like mitochondria and chloroplasts. DNA can be selectively stained with suitable dyes and its amount can be estimated by measuring the intensity of the stain. Such measurements have shown that the DNA content of the nuclei of the different cells of a given organism is always constant. Some specialised cells may have half or twice as much DNA. The multiples may be higher in some tissues, but they are usually whole numbers. This suggests that cells have one or two or more full sets of DNA instructions. Each full set of DNA instructions is called a *genome*. Each genome is packaged into one or more chromosomes. Cells with a single genome are said to be haploid, those with two are called diploid and those with more than two are known as polyploids. Cells with multiple sets of genomes have many identical sets of chromosomes — diploids with two sets of similar chromosomes and polyploids with many sets of similar chromosomes.

Although the importance of DNA in heredity and its mode of replication was not clear until the 1950s, the process of cell division and distribution of the nuclear material into the daughter nuclei was worked out as early as the 1880s. Details and exceptions have been added from time to time but the basic concept has remained unchanged. Rudolf Virchow suggested in 1859 that cells arise only from pre-existing cells—*Omnis cellulae cellula*. New cells arise as a result of the division of a pre-existing cell. It is this property of cells to divide which ensures that the set of characters is passed on from one cell generation to another. Each daughter cell receives from its parent a complete set of hereditary information and sufficient organelles to be able to carry out its metabolic reactions.

Cell division is of two types — mitosis and meiosis. Mitosis results in the multiplication of cells and during this process the daughter

cells contain the same number of chromosomes as their mother cells. Meiosis, on the other hand, halves the diploid genome and ensures an alternation of haploid and diploid generations. In diploid organisms, like higher plants and animals, meiosis occurs during the formation of gametes In haploid organisms, it occurs soon after the fusion of gametes (Fig 16 1).

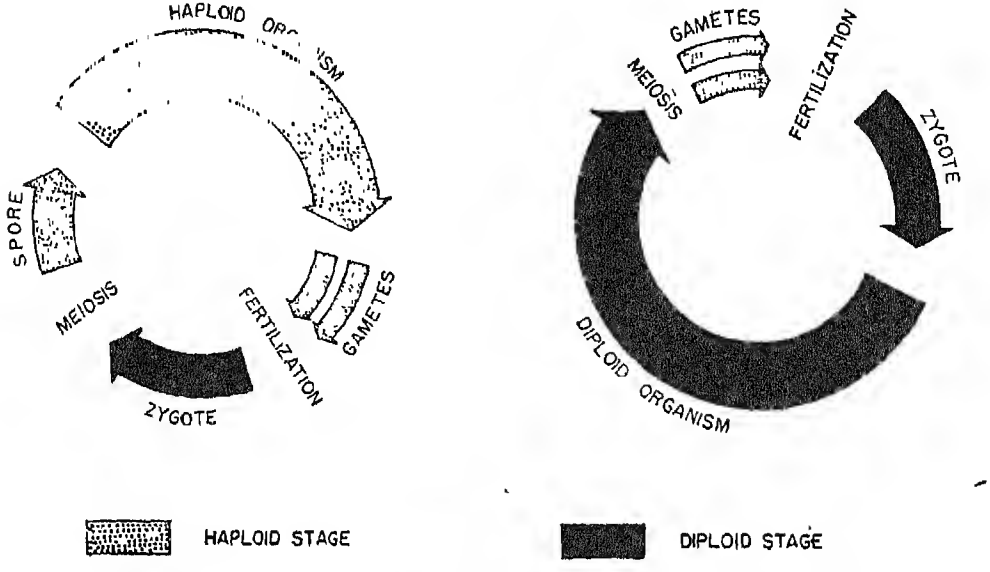


Fig 16 1 Stages at which meiotic divisions occur during the life cycles of haploid (*left*) and diploid (*right*) organisms

The sequence of events during the cell cycle is highly ordered and very precise. It involves three events: (1) DNA or genome replication, (2) nuclear division, and (3) cytokinesis or division of the cytoplasm. Cell division is a continuous and dynamic process.

Mitosis

Two identical nuclei are formed from one as a result of a mitotic division. Both the daughter nuclei contain the same amount of DNA and the same set of chromosomes and hereditary instructions as their parental cell. It occurs in vegetative or somatic cells. Mitosis, therefore, is also known as somatic or equational division. Before the onset of mitosis, when chromosomes become visible under optical microscopes, a cell is said to

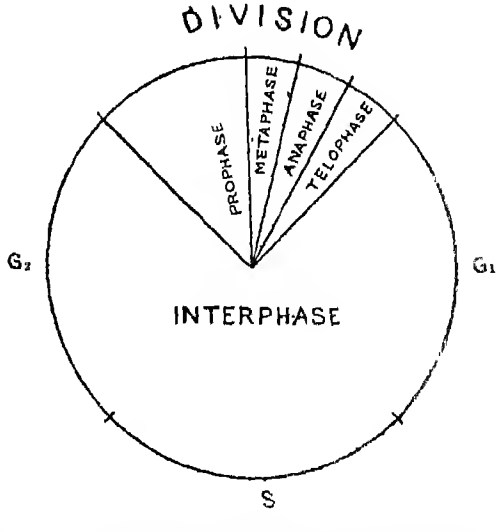


Fig. 16 2 The cell cycle showing the sequence of G_1,S,G_2 and M (mitosis or meiosis) phases.

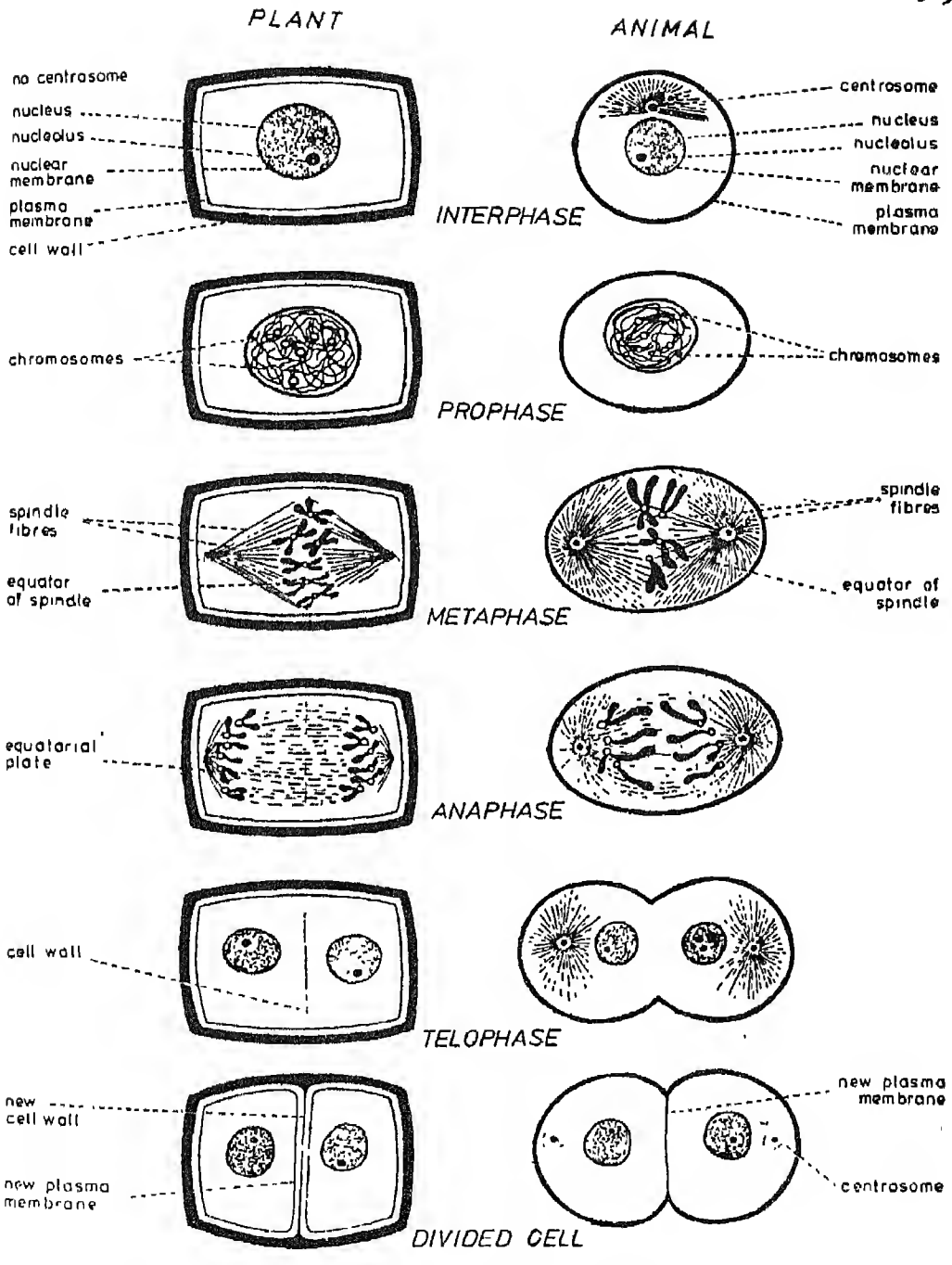


Fig. 16.3 Different stages of cell division in the plant and animal cells.

be in the interphase. An interphase intervenes between two cell divisions. It is during this period that the cell prepares to divide by duplicating its DNA and by synthesizing some macromolecules that will be required during mitosis. On the basis of biochemical studies, three distinct stages can be recognized during the interphase (Fig. 16.2) With the end of the cell division process, the cell enters the first stage of the interphase During this G_1 period (first growth or gap period), the nucleus regulates the growth of the cell. A lot of RNA and protein is synthesized during this period. This is followed by the S or synthetic phase during which the DNA is replicated. This replication results in doubling the number of DNA strands in each chromosome The S phase is followed by the second growth or gap period (G_2). During this period, the protein material and energy pools associated with the structure and movement of chromosomes are established. The G_2 phase is followed by the M phase during which chromosome complements are parcelled into the daughter nuclei The relative lengths of these phases differ in different organisms but are fixed for a given species under a given set of environmental conditions. The cells, which are not going to divide, do not proceed beyond the G_1 phase and start differentiating.

The M phase or mitosis (Fig. 16.3 and 16.4) is conventionally divided into four stages or phases. The first of these is called *prophase*. With the onset of prophase, chromosomes begin to coil tightly As coiling progresses, chromosomes turn thicker and shorter and, consequently, become visible under the light microscope. At this stage, the nucleus looks like a ball of wool. Due to the duplication that occurs during the preceding S phase, each chromosome during the prophase appears as a two-threaded structure. Each of these threads is called a *chromatid*. Both the chromatids of a chromosome are held together at a point which is called the *centromere* or the primary constriction The position of the centromere is

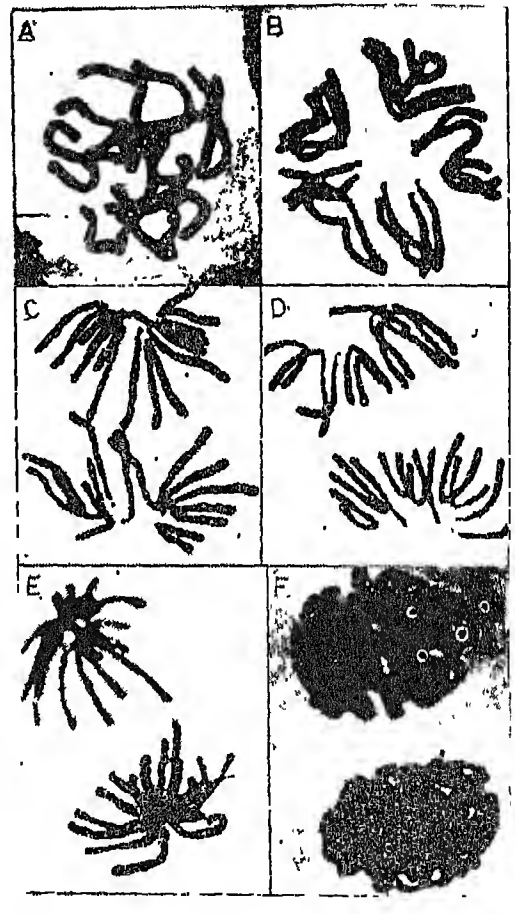


Fig 16.4 Some typical stages of mitosis in root tip cells of *Paris polyphylla* *A*. Prophase, *B*. Metaphase, polar view, *C*. Early anaphase, *D*. Late anaphase, *E*. Early telophase, *F*. Late telophase.
(*Courtesy*: Dr. Virendra Kumar)

characteristic of each chromosome. Some chromosomes have centrally located centromeres which divide the chromosomes into two equal arms (arbitrarily called left and

right arms). Others have almost terminal centromeres, thereby dividing the chromosomes into short (proximal) and long (distal) arms. Some chromosomes have secondary constrictions and these are the regions where nucleoli are attached. These regions are also known as nucleolar organizing regions. As the prophase progresses, the nucleolus gradually disappears and so does the nuclear membrane.

The complete disappearance of the nuclear membrane and the nucleolus heralds the beginning of the next stage of mitosis — metaphase. During this stage, chromosomes are thickest and shortest and become oriented in such a way that all the centromeres lie in one plane at the centre of the cell, forming a metaphase plate. The movement of chromosomes is guided by a series of fibres that arise from the nuclear and cytoplasmic material. The fibrillar structure is called the *spindle apparatus*. It is an arrangement of microtubules radiating from two poles at opposite ends of the metaphase plate. Chemically, they are composed of proteins which have large amounts of sulphur-containing amino acids. Some spindle fibers are attached at the centromeres of the chromosomes, while others lie between them. In most animal cells, each spindle pole contains a centriole which is also known as an aster (Fig. 16.3) Centrioles are fibrillar and starlike bodies. Chromosomes of diploid organisms can be matched into pairs. Members of each pair are of the same length and have the same centromere position.

The anaphase starts with the division of centromeres which results in the separation of sister chromatids along the long axis of the spindle to the opposite poles. The new chromosomes (formerly chromatids) appear to be pulled to the poles by their centromeres, while their arms point towards the metaphase plate. Consequently, a chromosome with a centrally located centromere will have a V-shaped configuration at this stage, while those with terminal and subterminal centromeres will look like I and J, respectively.

The telophase begins with the arrival of daughter chromosomes at the respective poles. During this period, chromosomes despiralise, lose their visible structural characteristics and become thin, long and granular. The nuclear membrane reappears and nucleoli are formed at the nucleolar organizing regions of relevant chromosomes. In the telophase, the events of the prophase are reversed and two nuclei are organized in each cell. The daughter nuclei then enter the G_1 stage of the next cell cycle.

Nuclear division is generally (but not always) accompanied by the parcelling of the cytoplasm into two daughter cells. This process is known as *cytokinesis* (Fig 16.3). In the animal cells, cytokinesis occurs by an invagination of the cell membrane almost in the middle of the cell. The furrow gradually deepens and ultimately divides the cell into two parts. In the plant cells, on the other hand, a cell plate is laid down at the equatorial plane This plate starts in the centre and extends laterally until it divides the cell into two parts. This is because the plant cells have a rigid cell wall. Repeated nuclear division, unaccompanied by cytokinesis, ultimately results in a multinucleate condition which is a permanent or temporary characteristic of some tissues like the endosperm of plant seeds or skeletal muscles.

Meiosis

Meiosis is characterized by two successive divisions of the cytoplasm and nuclei (meiosis I and meiosis II), accompanied by only one replication of the chromosomes. Meiosis in a diploid cell results in the formation of four haploid cells. It, thus, reduces the chromosome number as well as the amount of DNA

per cell to half. The interphase which precedes the onset of meiosis is similar to the interphase which precedes a mitotic division. There is the growth phase (G_1), followed by the S phase during which the DNA of chromosomes replicates. The S phase is followed by the second growth phase (G_2). Various stages of the interphase, meiosis I and meiosis II, are continuous processes and have been subdivided into many substages (Fig 16.5) only for the convenience of description.

Meiosis I or First Nuclear Division

Prophase I. The prophase of meiosis I is long lived as compared to the prophase of

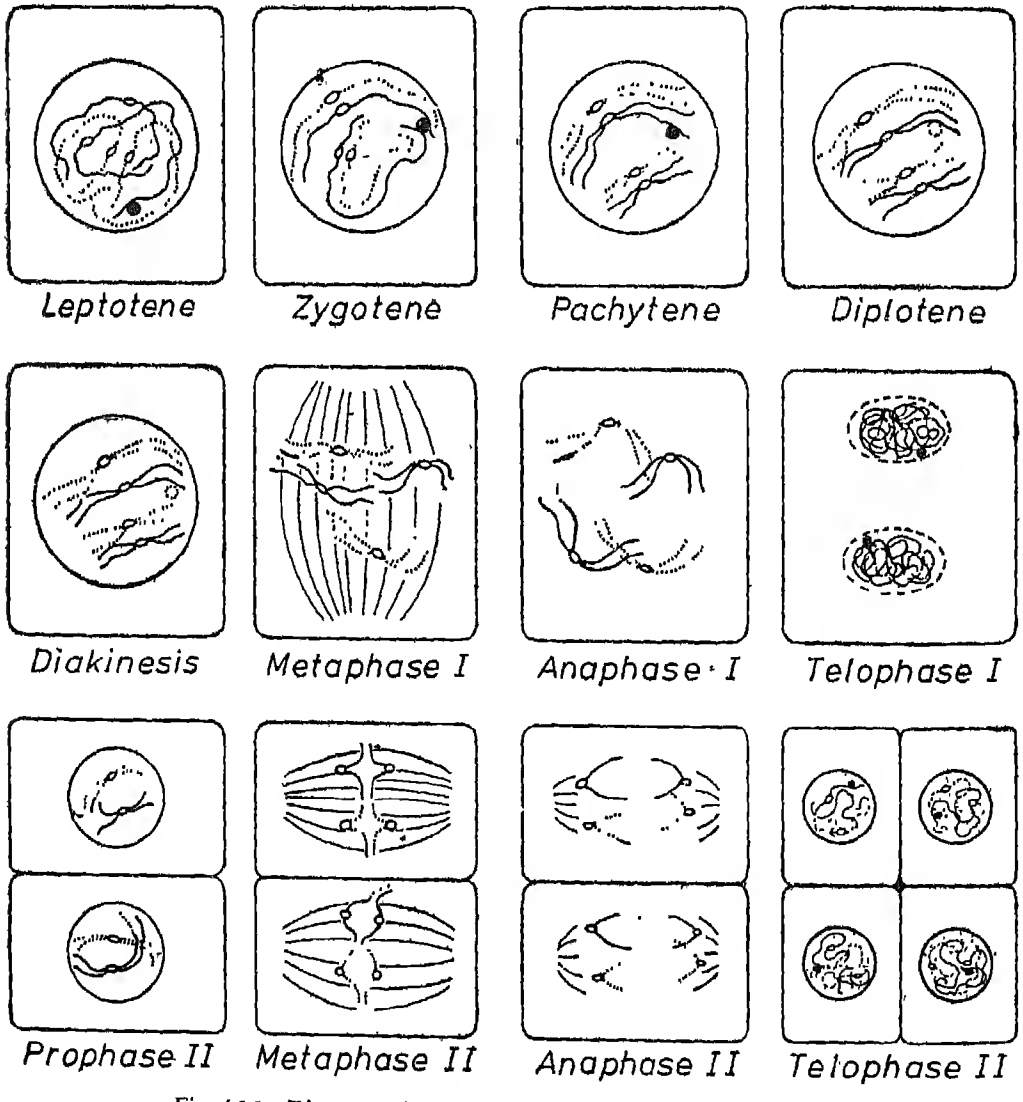


Fig 16.5 Diagrammatic representation of different stages of meiosis.

mitosis, and can be subdivided into five stages. During leptotene, chromosomes make their appearance as long and thin threads in the nucleus. As the prophase progresses, there is condensation, shortening and thickening of chromosomes. Each chromosome consists of two chromatids which are held together at the centromere region. Similar or homologous chromosomes start pairing during zygotene. During pachytene, the paired or synapsed homologues are quite clear because of shortening and thickening of chromosomes. Each paired unit is called a *bivalent* and consists of four chromatids. As diplotene starts, the synaptic forces between the paired homologues come to an end and the chromosomes start separating. Simultaneously, there is an exchange of parts of chromatids between homologous chromosomes (crossing-over) and this results in crosslike structures which are called chiasmata (singular : chiasma). With the progression of diplotene, the nuclear membrane and the nucleolus start disappearing. By diakinesis, the nuclear membrane and the nucleolus almost disappear. The bivalents become very short and, consequently, the chiasmata move towards the ends of chromosomes, away from the centromere, and ultimately slip out.

Metaphase I. The disappearance of the nucleolus and nuclear membrane and the organization of spindle fibres herald the beginning of the metaphase I and the end of the prophase I. The bivalents arrange themselves in the centre of the cell so that the chiasmata of the bivalents lie on the equatorial plate and the two centromeres of a bivalent on either side of it, pointing to the poles. The orientation of each bivalent is independent of the other and maternal and paternal chromosomes point towards the opposite poles.

Anaphase I. The centromeres of homologous chromosomes start moving along the spindle fibres towards the opposite poles, thereby dragging the respective chromosomes in the opposite directions. Due to the crossing-over during the prophase and the independent orientation of bivalents at the metaphase, the set of chromosomes that move to one pole consists of a mixture of paternal and maternal chromosome parts and chromosomes. Thus, a thorough mixing of maternal and paternal hereditary material occurs during meiosis.

Telophase I. The arrival of the chromosome sets at the opposite poles signals the beginning of the telophase. As only one partner of a homologous pair goes to one pole, only half the somatic or diploid number of chromosomes reaches either pole. Chromosomes uncoil and the nuclear membrane and the nucleolus are reorganized. Sometimes, the telophase I is absent and the anaphase I is followed by the metaphase II.

Interphase. The interphase between meiosis I and meiosis II is brief, if any. Generally, it is absent.

Meiosis II or Second Nuclear Division

Meiosis II is very much similar to mitosis.

Prophase II. Sometimes, the prophase II is absent and the anaphase I is followed by the metaphase II. Whenever it is present, it is short-lived and during this stage there is shortening of chromosomes and gradual disappearance of the nucleoli and the nuclear membrane.

Metaphase II. The beginning of the metaphase II is marked by the disappearance of the nuclear membrane and the nucleolus and the appearance of spindle fibres. The chromosomes arrange themselves on the equatorial plate and then centromeres divide. Thus, the sister chromatids of a chromosome get separated.

Anaphase II Sister chromatids separate and are dragged by their centromeres towards the opposite poles.

Telophase II. This stage starts with the arrival of chromosome complements to the respective poles. During this stage, the chromosomes despiralise and elongate, the nucleoli are organized and the nuclear membrane reappears

Cytokinesis. Division of the cytoplasm can occur after each nuclear division (meiosis I and meiosis II) or can be deferred until four nuclei are formed. In any case, details of the division process are the same as after mitosis

Like mitosis, meiosis, too, is a dynamic process and its one stage merges into another, without any sharp demarcation between them. As a result of meiosis I and meiosis II, the amount of DNA per nucleus as well as the chromosome complement are reduced to half that of the parental cell This is of essential for the formation of gametes, two of which unite to restore the diploid chromosome number of a typical organism.

DIFFERENCES BETWEEN MITOSIS AND MEIOSIS

Mitosis	*Meiosis*
Occurs in somatic cells.	Occurs in reproductive cells
Each DNA or chromosome replication is followed by one nuclear division, thereby maintaining the amount of DNA and the number of chromosomes per cell constant from generation to generation	Each DNA or chromosome replication is followed by two successive divisions of the nucleus Thus, each of the daughter cells contains half as many chromosomes and half as much DNA as its parent cell.
All chromosomes behave independently of each other	Homologous chromosomes get paired together.
The chromosomes at the metaphase are arranged in such a way that the centromeres lie at the metaphase plate and the arms of chromosomes are free	The chromosomes at the metaphase are arranged in such a way that the centromeres of homologous chromosomes lie on either side of the metaphase plate, pointing towards the opposite poles.
Generally speaking, there is no crossing-over or exchange of parts of chromatids between homologous chromosomes.	Crossing-over or exchange of parts of chromatids between homologous chromosomes is a rule, rather than exception
The time taken for the mitotic division is much shorter as compared to the time taken for the meiotic division in the same organism.	The time taken for the meiotic division is much longer as compared to the time taken for the mitotic division in the same organism.
As a result of mitosis, two daughter nuclei are produced from a single parental nucleus.	As a result of meiosis, four daughter nuclei are produced from a single parental nucleus.
Centromeres divide, thereby separating the two chromatids	Centromeres do not divide during the metaphase I Homologous chromosomes rather than chromatids separate. Centromeres divide during the metaphase II.

Unusual Methods of Division

In many simple and primitive organisms, the process of mitosis is not that elaborate. For example, in *Amoeba* or yeast, the nucleus divides by furrowing, without the appearance of chromosomes.

Sometimes, nuclei start dividing but do not complete this process. This results in the replication of DNA as well as the chromosomes but no division of the nucleus, thereby increasing the somatic number of chromosomes per cell. Such a process is known as *endoploidy* or *endoduplication*. Sometimes, the chromatids replicate but fail to separate, thus forming chromosomes with more than two chromatids. In the salivary gland chromosomes of *Drosophila* and of some other invertebrates, this type of polyteny may result in as many as 1000 chromatids per chromosome.

At the time of cytokinesis, the cytoplasmic organelles, like mitochondria, chloroplasts, centrioles and basal granules, get parcelled into daughter cells. Sometimes, instead of mature organelles, only their precursors are included in the daughter cells This is so because each of these organelles arises only from a pre-existing one. They have been shown to possess their own hereditary material. These organelles are at least partially self-reproducing units and their growth, differentiation and function are ultimately controlled by the nucleus.

EXERCISES

1. What is a genome? How many genomes are there in a haploid and in a diploid?
2. Why is cell division necessary?
3. Draw the life cycles of typical haploids and diploids, indicating the stages when meiosis occurs.
4. What are the events that occur during the interphase for the preparation of nuclear division?
5. Draw a labelled cell cycle.
6. With the help of self-explanatory labelled sketches, indicate the various stages of mitosis.
7. Draw self-explanatory labelled sketches of the various stages of the prophase I.
8. Describe briefly the structure and composition of the spindle apparatus in plants and animals.
9. How does cytokinesis of a typical plant cell differ from that of a typical animal cell.
10. Enumerate the various stages of meiosis.

11. List the differences between mitosis and meiosis
12 What is polyteny? Where do you expect to see a polytene chromosome?

CHAPTER 17

Principles of Inheritance

THE MECHANISM of inheritance was discovered long before DNA was identified as a carrier of genetic information and even before chromosomes were known. The way in which characters are transmitted from one generation to another was first demonstrated by Gregor Johann Mendel in 1866. He suggested that each cell of an organism contains two factors for each character, both of which separate and are passed on to different progeny through different gametes. This factor was later called a gene and genes were found to be located in chromosomes within the nuclei. Now, of course, we know in great depth about the physical and chemical organization of genes and also about the way in which they control the expression of characters in an organism. The foundation of genetics, the science of heredity and variation, however, was laid by Mendel over a century ago. It is, therefore, not inappropriate that he is known as the father of genetics.

Mendel (Fig. 17.1) grew up in what is now Czechoslovakia and was ordained a priest in a monastry. He carried out breeding experiments with garden peas (*Pisum sativum*) for about nine years and summarized the results to elucidate the mechanism of inheritance. Many people had carried out breeding experiments in a variety of plants and animals before Mendel but none of them were able

Fig. 17.1 Gregor Johann Mendel — the father of genetics.

to analyse the results as clearly as Mendel could Even in garden peas many people had carried out breeding experiments and had observed a blending of parental characters in the offspring. Similar breeding experiments were conducted by Mendel and he took advantage of his training in mathematics and science for analysing his results Unlike his predecessors, he concentrated on the inheritance patterns of only a few sets of characters which were sharply contrasting in the parents He pooled the data of many similar crosses and analysed the results statistically. He considered the inheritance of one character at a time in the beginning and later of two or more characters. Separate and systematic recording of data of each year was another key to his success. The choice of the garden pea as the experimental material was also a wise one because its flower structure facilitates controlled breeding, many generations are produced in a short time and the plants are easy to cultivate Moreover, a large number of pure varieties with distinct contrasting characters were available commercially. The petals of a pea-flower completely enclose the reproductive organs until fertilization, thereby ensuring self-pollination or the fusion of male and female gametes from the same plant At the same time, cross-pollination or fusion of male and female gemetes from different plants can be achieved by removing the anthers from a flower before pollen grains mature and by bringing about pollination by dusting the stigma with pollen from a desired plant

After a careful examination of a large number of characteristics of the pea plant, Mendel decided to work and report on the inheritance patterns of seven characters. Each of these seven characters occurred in two contrasting forms (Table 17.1). Thus, the plants were either tall or dwarf, flowers were either red or white, etc Mendel started his experiments with plants which were true-breeding for the characters that he studied. For this, he allowed the plants to produce progeny as a result of self-fertilization and selected only those which produced only one type of progeny for a number of successive generations. Plants which gave rise to parental as well as new types were discarded by him. Selection of pure-breeding strains as the starting experimental material was important for the successful completion and interpretation of Mendel's experiments.

Once the pure lines were established, Mendel performed many crosses by dusting the pollen from the pea plants with one character onto the stigmas of plants with the contrasting character. For example, he pollinated tall plants with the pollen from

TABLE 17.1

Dominant and Recessive Characters of the Garden Pea that were Studied by Mendel

Character studied	*Dominant*	*Recessive*
Plant height	Tall (2 metres)	Dwarf ($\frac{1}{4}$ metre)
Flower and pod position	Axial	Terminal
Pod colour	Green	Yellow
Pod shape	Non-constricted or full	Constricted
Seed coat or flower colour	Coloured	White
Seed shape	Round	Wrinkled
Endosperm colour	Yellow	Green

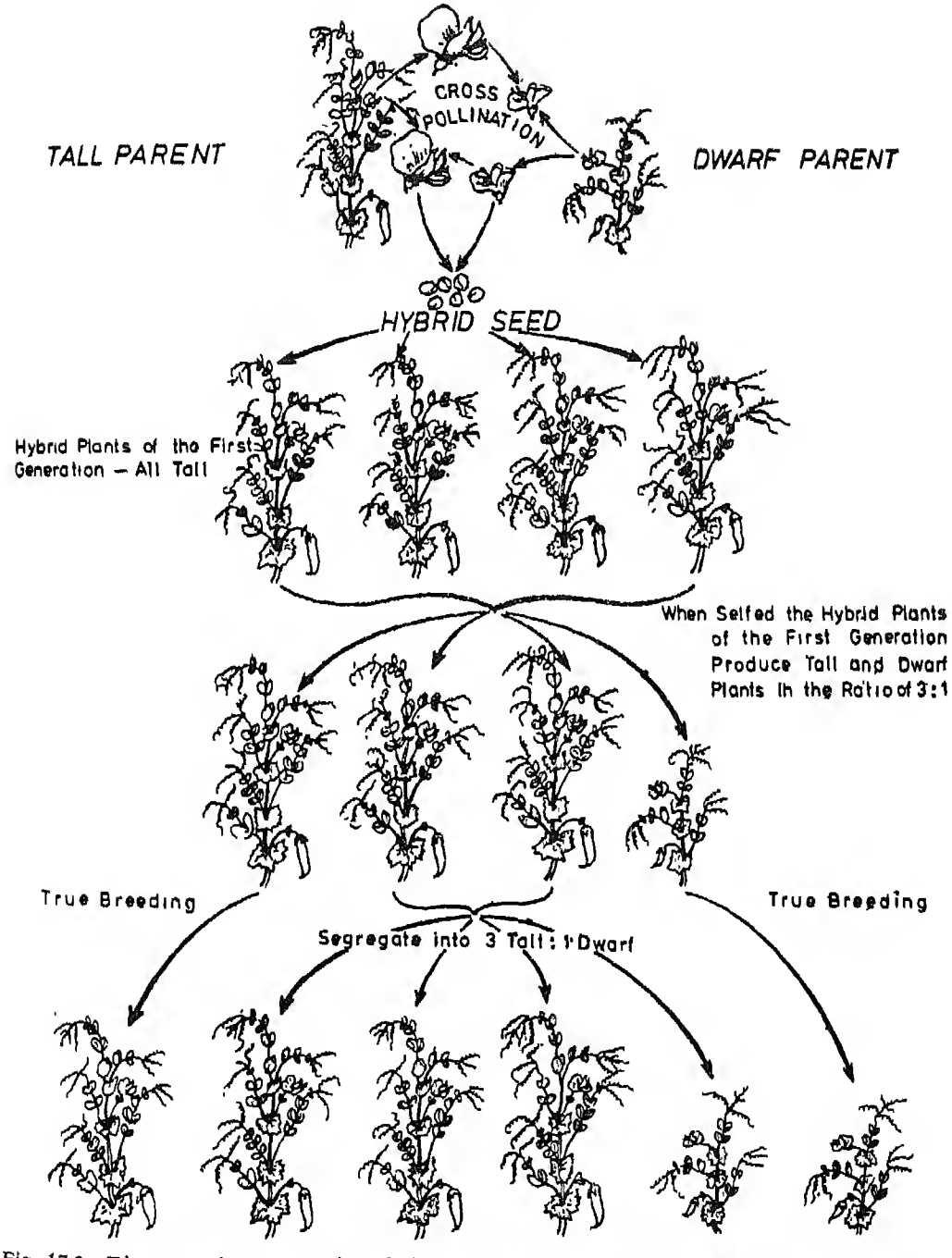


Fig 17.2 **Diagrammatic representation of Mendel's results obtained by crossing tall plants with the dwarf ones.**

dwarf plants or vice versa. Mendel found that all the progeny of the first filial (daughter) generation (F_1 generation) resembled one of the parents and during this generation there was no trace of the character of the other parent. Thus, in a cross between the true-breeding tall and the true-breeding dwarf plants, only tall plants were produced in the F_1 generation. The results were the same, irrespective of whether the tall or the dwarf parent was the source of pollen. In other words, reciprocal crosses gave identical results.

Principle of Dominance

In his first major publication, Mendel described the results of seven pairs of contrasting characters. He noticed that one character from each of the contrasting pairs dominates over the other and is expressed in the F_1 generation, whereas the other character is not expressed and remains recessive (Table 17.1). In modern terminology, the pair of contrasting characters is known as the *allelic pair* and each member of such a pair is called an allele of the other. Thus, round and wrinkled is an allelic pair and round is the allele of wrinkled and vice versa. By definition, alleles are the alternative states of the same gene. There may be more than two alleles of a particular gene.

Mendel allowed the F_1 plants to pollinate themselves and raised the second filial (F_2) generation. He noticed that although there is no trace of the recessive character in the first generation, it makes its appearance in the second generation (Fig. 17.2). Thus, the recessive character is not lost. It is simply not expressed in the presence of its dominant allele. A numerical analysis of the F_2 progeny revealed that three quarters (3/4) of them expressed the dominant character and the remaining one quarter (1/4) expressed recessive trait (Table 17.2). For example, when the F_2 generation was raised from the tall and dwarf plants, Mendel obtained a total of 1064 plants, 787 of which were tall and 277 dwarf. All crosses yielded similar results. The ratio of plants with the dominant character to the plants with the recessive character was always close to 3 : 1.

A cross made to study the pattern of inheritance of a single pair of alleles is known as a *monohybrid cross* and the ratio obtained is called the *monohybrid ratio*. Mendel performed seven different monohybrid crosses and in each case obtained a monohybrid ratio of 3 · 1 in the F_2 generation.

Principle of Purity of Gametes

Mendel raised F_3 and subsequent generations and obtained expected results. The plants which exhibited the recessive character in the F_2 generation bred true, i.e, their progeny maintained their characters from generation to generation. But, out of the plants which exhibited the dominant character, one-third turned out to be true-breeding and two-thirds yielded offspring which displayed the dominant and recessive characters in the ratio of 3 : 1 (Fig. 17.2). For example, among 565 plants which were raised from round seeds of the first generation, 193 yielded only round seeds, whereas 372 produced both round and wrinkled seeds in the ratio of 3 : 1. Thus, the 3 : 1 F_2 ratio is actually a 1:2 1 ratio in which the first and the last groups are true-breeding for dominant and recessive characters, respectively. Fifty per cent of the total progeny, belonging to the middle group, are hybrid, containing both dominant and recessive characters, but express only the dominant one.

On the basis of these results, Mendel postulated that each gamete produced by the parent plant contains one factor for one character. A diploid individual carries two factors for each character in each of its cells.

TABLE 17 2

Mendel's Results of Monohybrid Crosses in the Garden Pea

Parental cross	F_1 *progeny*	F_2 *progeny*	F_2 *Ratio*
Round × wrinkled seeds	All round	5,474 round 1,850 wrinkled 7,324 total	2.96 : 1
Yellow × green seeds	All yellow	6,022 Yellow 2,001 green 8,023 total	3.01 : 1
Coloured × white flowers	All coloured	705 coloured 224 white 929 total	3.15 : 1
Inflated × constricted pods	All inflated	882 inflated 299 constricted 1181 total	2.95 : 1
Green × yellow pods	All green	428 green 152 yellow 580 total	2.82 : 1
Axial × terminal flowers	All axial	651 axial 207 terminal 858 total	3.14 : 1
Tall × dwarf plants	All tall	787 tall 277 dwarf 1064 total	2.84 : 1

Each factor maintains its identity through successive generations and does not mix with others. Mendel used letters of the alphabet as symbols for the factors — capital letters to symbolize the dominant genes and small letters to symbolize the recessive ones. We can designate the factor for red flowers as *C* and the factor for white flowers as c A true-breeding red-flowered plant will carry two dominant factors (genes) for redness (*CC*), and the white-flowered plant will carry two recessive genes for whiteness (*cc*). Each gamete produced by the true-breeding red-flowered parent will carry only one *C* gene, whereas each gamete from the white-flowered parent will carry only one *c* gene. Fusion of these two types of gametes will result in a zygote with one gene of each kind (*Cc*), irrespective of whether the dominant or the recessive parent is the source of pollen. Plants with both the alleles will produce two types of gametes, 50 per cent carrying the dominant allele and 50 per cent carrying the recessive allele. Individuals having two different alleles of the same gene (i.e., *Cc*) are known as heterozygotes or to be heterozygous as they are capable of producing two different types of gametes. . Individuals, in which both the alleles of a gene are similar (for example, *CC* or *cc*), are known

to be homozygous. Such individuals are capable of producing only one type of gamete and, therefore, on selfing or on being fertilized by a similar gamete, produce the parental type of progeny in successive generations. In other words, they are true-breeding. Homozygous dominant and heterozygous individuals look alike because in the latter case the dominant allele dominates over the recessive one. Thus, often, there is no one-to-one correspondence between the different possible genetic constitutions (genotypes) and the possible appearances (phenotypes). For example, in the F_2 progeny of the cross between pure red- and pure white-flowered plants, there are only two phenotypes (red and white) but three possible genotypes (*CC*, *Cc* and *cc*). The phenotypic ratio in F_2 is 3 red: 1 white, but the corresponding genotypic ratio is 1 *CC*: 2*Cc* : 1 *cc*. Genetic representation of one of many monohybrid crosses performed by Mendel is given in Fig. 17.3.

Principle of Segregation

In order to explain the mode of inheritance of characters through successive generations, Mendel proposed the idea of segregation of

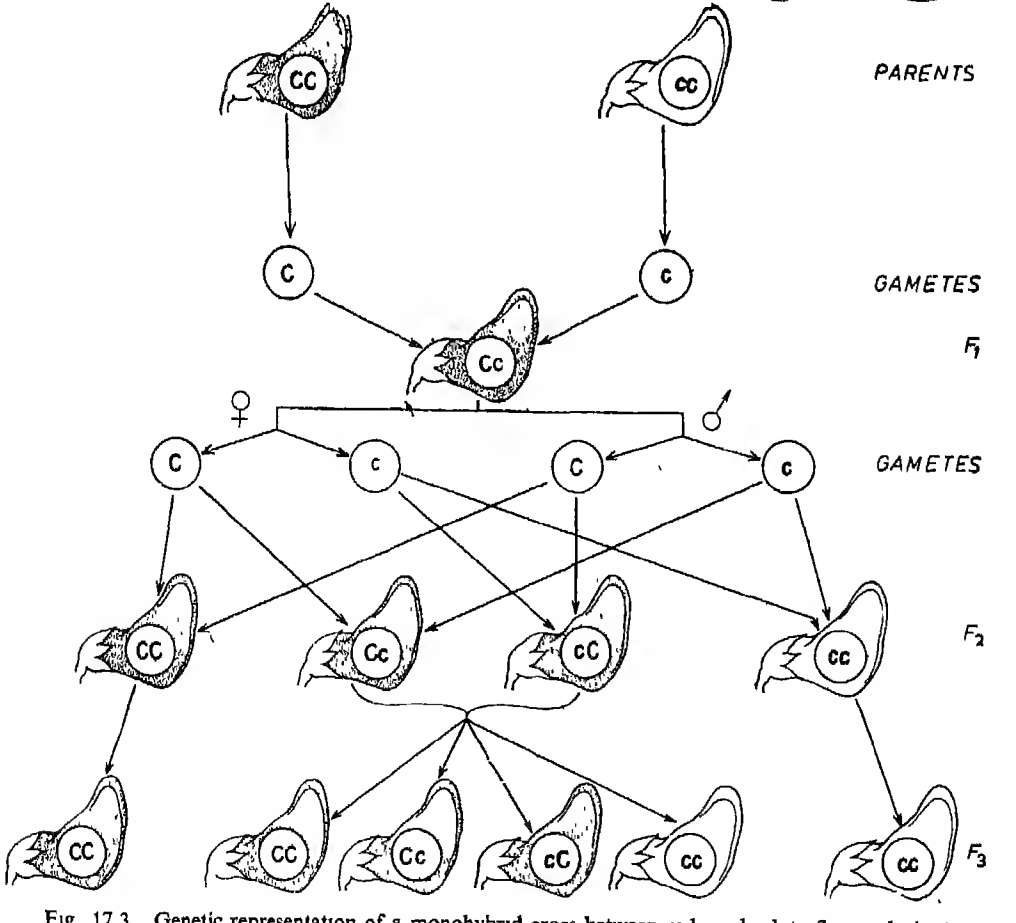


Fig 17 3 Genetic representation of a monohybrid cross between red- and white-flowered plants.

factors during reproduction and their coming together during fertilization He suggested that the two alternative factors for each character become separated during the formation of gametes and each factor has an equal chance of being transferred to the offspring. Mendel took advantage of his training in statistics and used the law of probability to explain his results. Let us consider the cross between a tall (*TT*) and a dwarf (*tt*) plant (Fig. 17.4) in the light of the probability theory. Since each parent can contribute only one kind of factor to his offspring, the F_1 progeny must all be of the same genotype (*Tt*). For the F_2 generation, each parent is *Tt* and, therefore, the offspring can receive either factor (*T* or *t*) from each parent. Depending upon which factor is received from which parent, the four possible combinations will be as follows:

T from male parent, *T* from female parent — *TT*

T from male parent, *t* from female parent — *Tt*

t from male parent, *T* from female parent — *Tt*

t from male parent, *t* from female parent — *tt*

Each offspring has an equal chance of receiving either *T* or *t* from each parent. Therefore, the probability of receiving *T* is ½, and the probability of receiving *t* is ½. The probability of receiving a particular combination of factors is simply the product of these individual probabilities. The probabilities of the four types of F_2 progeny, therefore, will be the following:

$TT = \frac{1}{2}T \times \frac{1}{2}T = \frac{1}{4}$

$Tt = \frac{1}{2}T \times \frac{1}{2}t = \frac{1}{4}$

$Tt = \frac{1}{2}t \times \frac{1}{2}T = \frac{1}{4}$

$tt = \frac{1}{2}t \times \frac{1}{2}t = \frac{1}{4}$

Of these, the first three classes will be phenotypically similar, whereas only the two classes in the middle will be genotypically alike. Thus, the F_2 phenotypic ratio will be tall: dwarf = 3 : 1 and the F_2 genotypic ratio will be homozygous dominant (*TT*): heterozygous (*Tt*): homozygous recessive (*tt*) = 1 : 2 : 1. It is clear from these considerations that the members of each pair of alleles segregate from each other during the formation of gametes and are randomly transferred to the progeny.

Principle of Independent Assortment

Besides emphasizing the phenomena of purity of gametes, dominance and recessiveness of characters and factors, and segregation of alleles during the formation of gametes, Mendel proposed the concept of independent assortment. He suggested that the inheritance of one factor is unaffected by the inheritance of the other. In other words, each gene is assorted independently of the other during its passage from one generation to the other. Mendel arrived at this conclusion after analysing the results of crosses between plants which differed in two characters, rather than in only one. Crosses involving two characters are known as dihybrid crosses, in contrast to a monohybrid cross in which only one character difference is involved.

Mendel crossed pure-breeding round and yellow-seeded (*RRYY*) plants with pure-breeding wrinkled and green-seeded (*rryy*) ones. All the F_1 seeds were round and yellow (*RrYy*), as expected, on the basis of the dominance and purity of gametes. The F_2 seeds were of four types and the numbers of each type were as follow:

Round and yellow	=	315
Wrinkled and yellow	=	101
Round and green	=	108
Wrinkled and green	=	32

A closer analysis of these results reveals that Round: Wrinkled = 423 : 133 or 3 : 1, and Yellow : Green = 416 : 140 or 3 : 1.

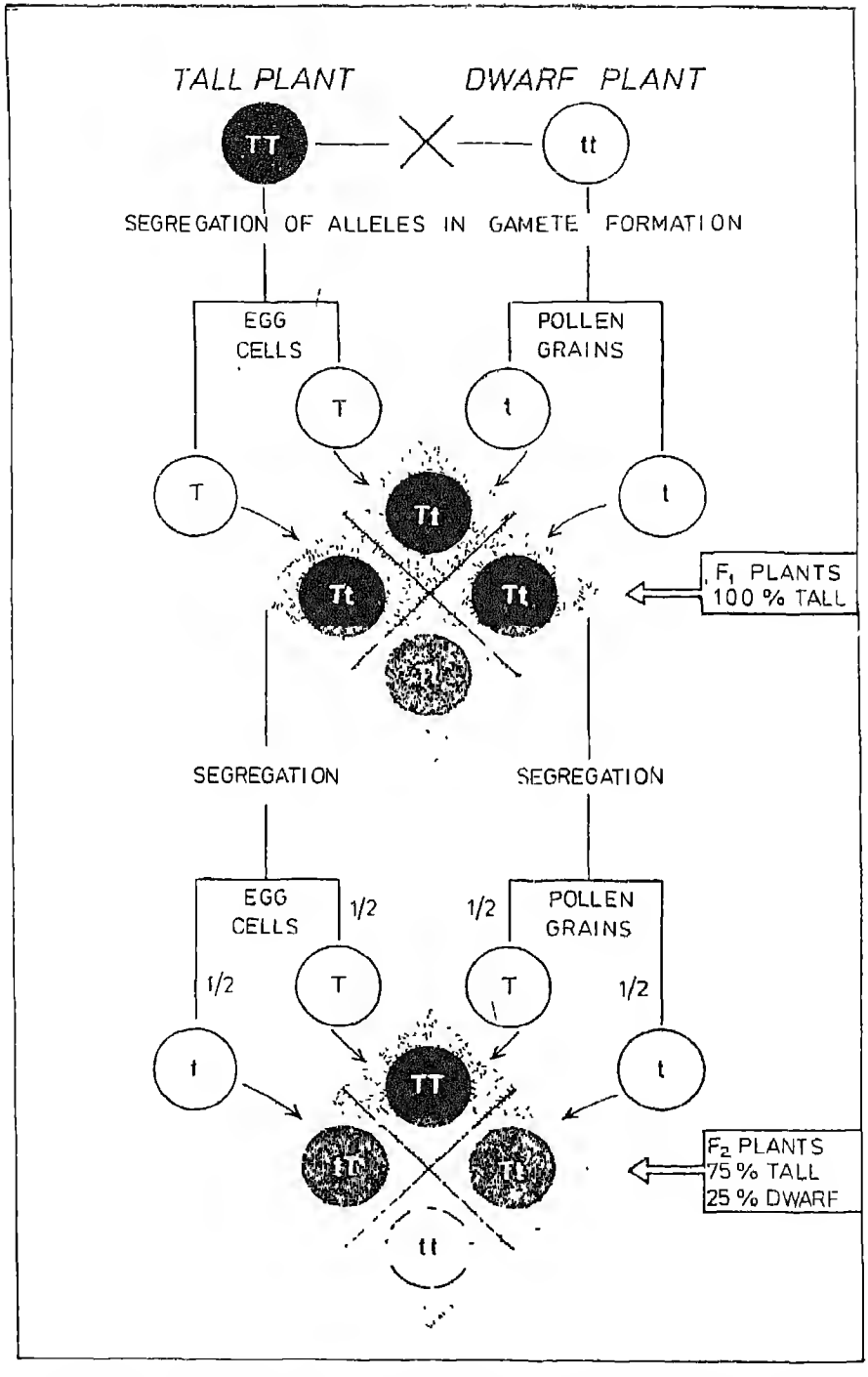


Fig. 17.4 Diagrammatic representation of a cross between tall and dwarf plants, showing segregation of alleles at the time of formation of gametes

Three-fourths of the round seeds are yellow and one-fourth green. Similarly, three-fourths of the wrinkled seeds are yellow and one-fourth green In other words, three-fourths of the yellow seeds are round and one-fourth wrinkled, whereas three-fourths of the green seeds are round and one-fourth wrinkled. If we consider both the characters the following are the expected and observed frequencies on the basis of independent assortment of factors·

Round and yellow = ¾ Round = ¾ Yellow = 9/16 of the total --315

Wrinkled and yellow = ¼ Wrinkled × Yellow = 3/16 of the total — 101

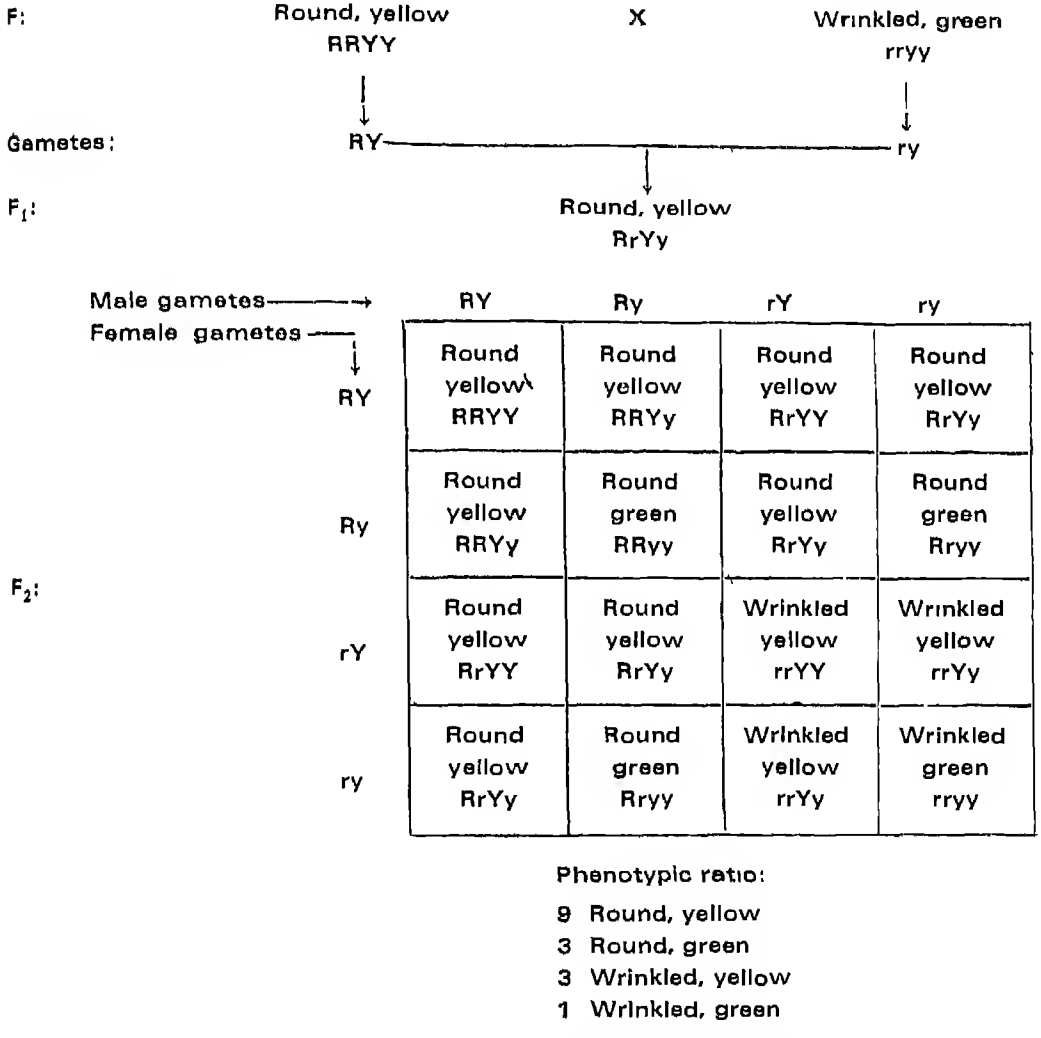


Fig 17 5 Results of a dihybrid cross between pure-breeding plants with round, yellow and wrinkled green seeds and Mendel's explanation of the mode of inheritance.

Round and green = ¾ Round × ¾ Green
= 3/16 of the total —108
Wrinkled and green = ¾ Wrinkled × ¾
Green = 1/16 of the total — 32

Thus, a dihybrid cross yields four phenotypes in the ratio of 9 : 3 : 3 : 1. It can be represented as a product of two monohybrid crosses:

(3 Round + 1 Wrinkled) (3 Yellow + 1 Green) = 9 Round and Yellow + 3 Round and Green + 3 Wrinkled and Yellow + 1 Wrinkled and Green.

These findings and explanations agree quite well with the law of probability which states that the frequency of coincidence of two or more events is equal to the product of the frequencies of independent events. One of Mendel's dihybrid crosses with gene symbols is presented in Fig. 18 5. Mendel performed a trihybrid cross (a cross in which the parents differed in three characters) and obtained results predicted on the basis of independent assortment of factors

Mendel's hypotheses about the inheritance of factors agree quite well with what we now know about chromosomes, mitosis, meiosis, DNA and genes. A diploid organism possesses two full sets of chromosomes and mendelian factors (genes) Each allele is contained in one member of a homologous pair of chromosomes. During meiosis, at the time of gamete formation, the members of a homologous pair of chromosomes separate and so do the pair of factors which control a character One allele and one chromosome of each pair are contributed through each of the gametes and, thus, the diploid number of chromosomes and the pair of factors are restored. The factors are located on chromosomes, the hereditary material of which is DNA Each homologous pair of chromosomes assorts independently of the other and so do the factors

Although Mendel's experiments and conclusions are the foundation stones of the science of genetics, their importance was not realized during Mendel's days. The experimental results were published in 1866 but no one paid any noticeable attention to these until three scientists, Hugo de Vries, Tschermak and Correns, working independently of each other and unaware of Mendel's findings, arrived at the same conclusions in the beginning of the present century. They came across Mendel's publication while hunting the literature and realized its importance. These scientists are known as rediscoverers of mendelism

EXERCISES

1. Why Mendel is known as the father of genetics?
2. Why did Mendel select the pea plant for his experiments on plant hybridization?
3. What will you get in F_1 and F_2 generations in the following crosses?
   (*a*) Pure tall × pure tall
   (*b*) Pure tall × pure dwarf
   (*c*) Heterozygous tall × pure tall
4. Explain the following terms.
   (*a*) Allele, (*b*) Genotype, (*c*) Phenotype, (*d*) Heterozygote, and (*e*) Homozygote

5 Prepare a diagrammatic account of a dihybrid cross
6. State the principles of inheritance that were discovered by Mendel.
7 What is the meiotic basis of independent assortment?
8. Who were the rediscoverers of mendelism?
9. Why Mendel was crowned with success, whereas his predecessors failed to discover the basic principles of inheritance?

CHAPTER 18

Linkage and Crossing-over

MENDEL'S IDEA of the purity of gametes, the dominance of one allele over the other and the segregation of alleles still holds good. But the principle of independent assortment has been slightly modified This principle states that if we consider the inheritance of two or more factors at a time, their distribution in the gametes and progeny of subsequent generations is independent of each other Soon after the rediscovery of mendelism, it was realized by Bateson and Punnet that there are only seven pairs of chromosomes in *Pisum sativum* (garden pea) which could show independent assortment But there must be a large number of factors or genes that determine the various characters of this plant. If the genes are located on chromosomes, there must be many genes on each chromosome The genes situated on the same chromosome should not show independent assortment. On the other hand, they should be inherited together This fact was demonstrated by Morgan in 1910 as a result of breeding experiments in the fruit-fly (*Drosophila melanogaster*).

Drosophila melanogaster is a very suitable organism for genetical experiments because it can be grown in large numbers under laboratory conditions, its generation time is only 10-12 days (as contrasted to one year in *Pisum sativum*), a large number of progeny are produced after each mating The unequivocal proof of the location of the genes on chromosomes came from the experiments carried out with the fruit-flies by Morgan (1910) and Bridges (1916). Their task was made easier by the findings of earlier scientists that each cell of *Drosophila* has four pairs of chromosomes and that in males the two members of one of the four pairs are morphologically dissimilar (Fig. 18.1) The three pairs of chromosomes, which are similar in male and female flies, are known as *autosomes*. The members of the fourth pair are known as *sex-chromosomes*. Male fruit-flies have heteromorphic sex-chromosomes — X and Y. Females have two X-chromosomes. Human beings also have 22 pairs of autosomes and one pair of sex-chromosomes — XX in females and XY in males. Thus, in fruit-flies as well as in human beings, all the eggs produced by females contain one X-chromosome each. But 50 per cent of the sperms produced by males carry X-chromosomes, whereas the other 50 per cent carry Y-chromosomes.

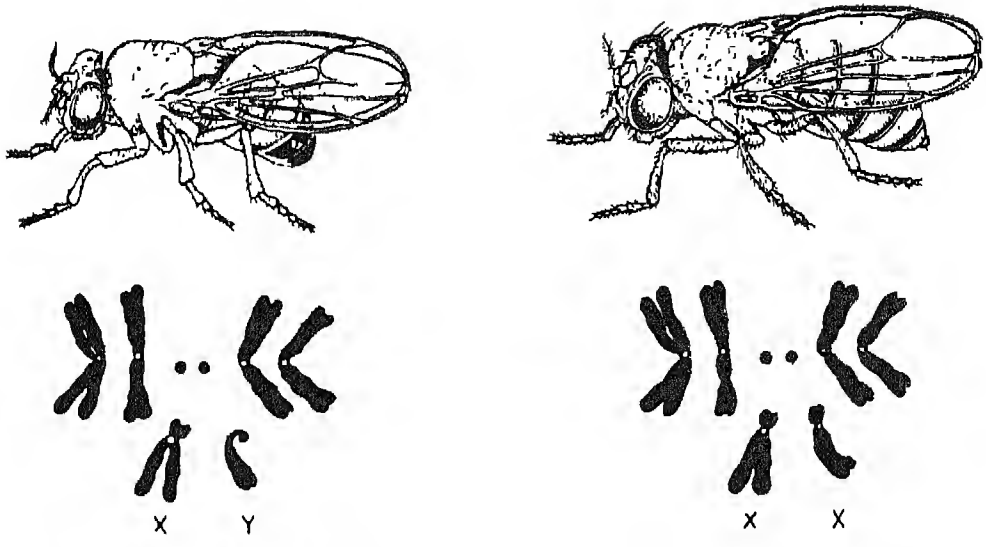


Fig. 18.1 Male (*left*) and female (*right*) *Drosophila* flies with their chromosome complements (*below*)

Fertilization by the X-carrying sperm results in a XX-zygote, which develops into a female progeny. Fertilization by the Y-carrying sperm results in a XY-zygote, which develops into a male progeny.

The morphological difference between the X- and Y-chromosomes of males enables one to trace their passage to males and females of subsequent generations. The X-chromosomes of males are inherited by the females of the first filial generation and are passed on to the males of the second filial generation. In subsequent generations, they go from one sex to the other. This type of criss-cross inheritance is shown in Fig. 18.2 in which the passage of the male X-chromosome has been depicted up to the second generation. Morgan noticed that certain characters, like the colour of eyes in *Drosophila*, show criss-cross inheritance. Normal fruit-flies have red eyes. Morgan came across a fly which had white eyes. A cross between the red-eyed females and the white-eyed males yielded only the red-eyed progeny. But in F_2, 25 per cent of the total or 50 per cent of the male progeny were white-eyed. These results led Morgan to suggest that the gene for white eye colour in *Drosophila* is located on the X-chromosome (because both of them show criss-cross inheritance) and that the Y-chromosome of a male does not possess the allele of this gene, whereas the X-chromosomes of normal females do possess the dominant alleles which determine the red eye character. The F_1 females are heterozygous and, therefore, red-eyed. The results obtained by Morgan can be explained diagrammatically if we put the gene for the white-eye character (w) on the X-chromosome of the male parent and the gene for the red-eye character (W) on the X-chromosomes of the female parent. All the white-eyed flies of the second generation in Morgan's experiment were males (although all the males were not white-eyed). The white-eye character, therefore, was always linked to the male sex. Todate, about 150 sex-linked characters have been discovered in fruit-flies All these must be located on the X-chromosome. Haemophilia, colour blindness, and about 50 other

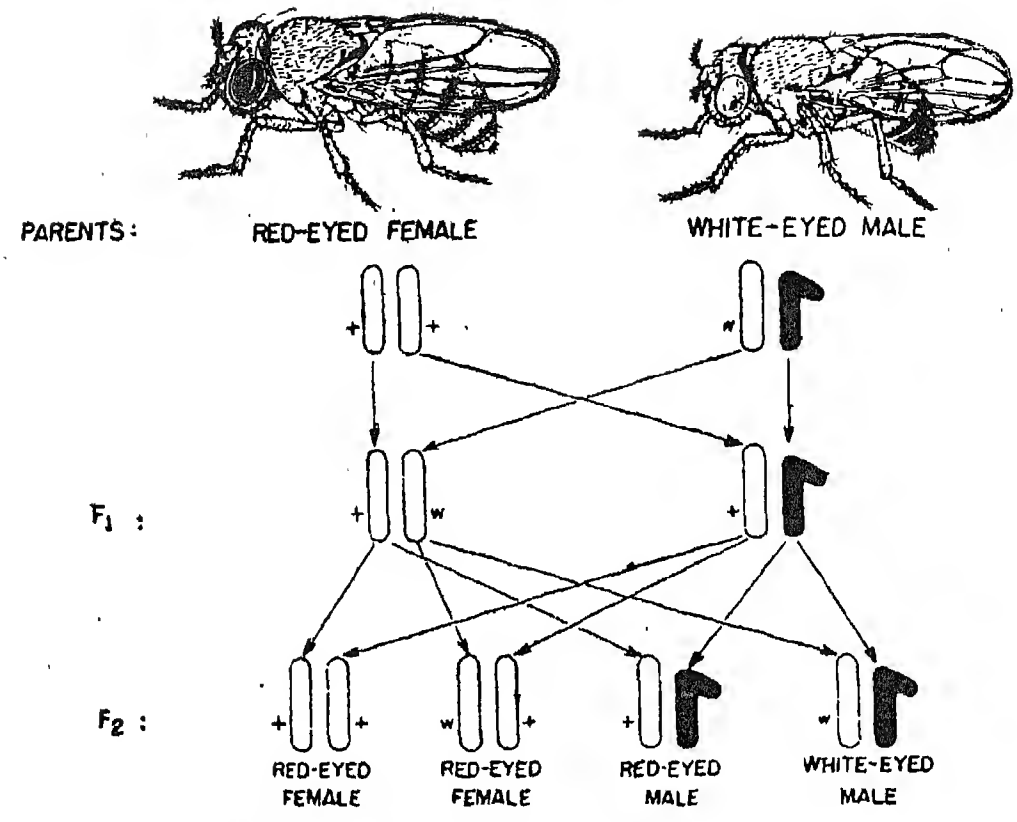


Fig. 18.2 Criss-cross inheritance without and with crossing-over from a heterozygous parent.

characters in human beings have been found to be sex-linked. C. B. Bridges, one of Morgan's students, provided further evidence to prove that genes are located on chromosomes. This was a very significant development in biology and exemplifies how researches in two diverse disciplines contribute to the development of a fundamental concept. Cytological observations of chromosomes and inheritance of characters were directly correlated by Bridges in 1916 and this heralded the beginning of the science of cytogenetics. Before this, cytology and genetics were developing as independent subjects.

Since there are many sex-linked characters and only one sex chromosome, all the sex-linked genes must be located on the chromosome that is associated with the determination of sex. The same is true of autosomes. Since there are far more genes than the number of chromosomes in an organism, many genes must be located on a single chromosome. All the genes located on the same chromosome are said to be linked together because they tend to get inherited together. Such a group of genes forms one linkage group. The number of linkage groups can be determined by breeding experiments and corresponds to the number of chromosomes which can be determined cytologically.

Breeding experiments carried out on *Pisum sativum* and *Drosophila* showed that even

linked genes do not always remain together in successive generations. Cytological observations have shown that during the prophase of meiosis I there is an exchange of parts of

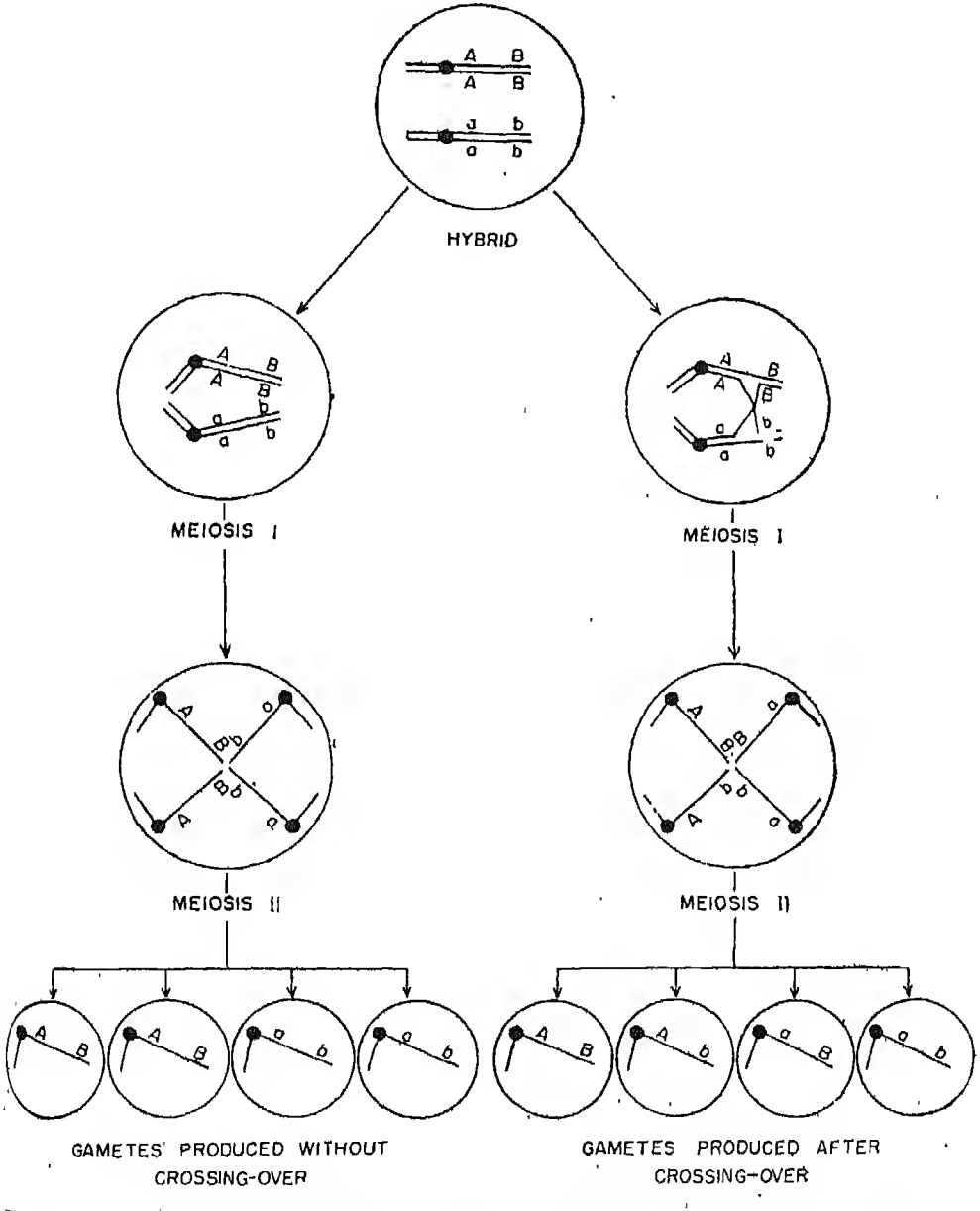


Fig. 18.3 Types of gametes produced without and with crossing-over from a heterozygous parent.

chromatids between homologous chromosomes This crossing-over results in an exchange of genes between maternal and paternal chromosomes. Consequently, besides the parental types, new types of gametes are produced These gametes have new combinations of genes on the same linkage group — some genes from the male and some from the female parent The consequences of crossing-over are diagrammatically represented in Fig. 18.3. Dominant genes *A* and *B* come from one parent and recessive genes *a* and *b* from the other parent. The hybrid is heterozygous and is capable of producing only two types of gametes (*AB* and *ab*) in the absence of a crossing-over between the two genes. On the other hand, four types of gametes (*AB*, *ab*, *Ab* and *aB*) are produced if there is a crossing-over between these two genes. Without crossing-over, genes *A* and *B* (or genes *a* and *b*) remain linked and are passed on together to the next generation. As a result of crossing-over, they get separated and go to different progeny. Thus, linkage and crossing-over are alternatives of each other. If the chance of linkage is the same as that of crossing-over, i.e., if crossing-over occurs in only 50 per cent of the cases, four types of gametes with equal frequencies (*AB*=25%, *ab*=25%, *Ab*=25% and *aB*=25%) are produced from a heterozygous individual. In such a situation, the genes show independent assortment even if they are on the same chromosome. Thus, there is independent assortment of genes under the following two situations: (1) If the genes are situated on different chromosomes, and (2) If the genes are on the same chromosome but far apart so that in 50 per cent of the gametes they get separated as a result of crossing-over.

Mendel was lucky in the choice of characters for his experiments because the seven characters studied by him were located on four different chromosomes and those which were on the same chromosome were very far from each other so as to allow their separation in 50 per cent of the gametes. Although these facts were not known to Mendel, they were responsible for the results which led to the formulation of the principle of independent assortment

If the genes located on the same chromosome are close together, the frequency of their separation is less than 50 per cent. Consequently, there is preponderance of the parental type progeny. In the extreme case, the two genes can be so close together as not to allow any crossing-over. In such a situation, only the parental type of progeny is produced. The farther the two genes are apart on a chromosome, the more likely is the occurrence of crossing-over between them. In other words, the frequency of crossing-over is an index of the relative distances of the genes on a chromosome or of the strength of linkage between them. The concept of crossing-over and linkage is based on the presupposition that genes are linearly arranged on a chromosome. The progeny with a new combination of characters (i.e., other than parental) is known as the recombinant type. They are produced as a result of crossing-over or recombination during the formation of gametes in the parents The frequency of crossing-over can be determined cytologically by counting the number of chiasmata formed during prophase I of meiosis. The recombination percentage can be calculated by determining the frequencies of the parental and recombinant types of progeny. It should be borne in mind that each crossing-over (or chiasma) results in two parental and two recombinant types of gametes (Fig. 18.4). This is so because at a particular cross-over point, only two chromatids are involved in exchanges of their parts and the other two are not. Thus,

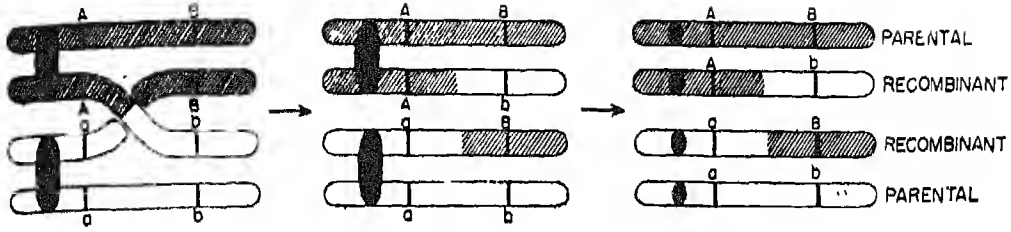


Fig 18 4 Diagram to show that crossing-over results in 50 per cent parental and 50 per cent recombinant types of gametes.

in order to produce 50 per cent recombinant gametes there should be crossing-over in all the mother cells that undergo reductional division.

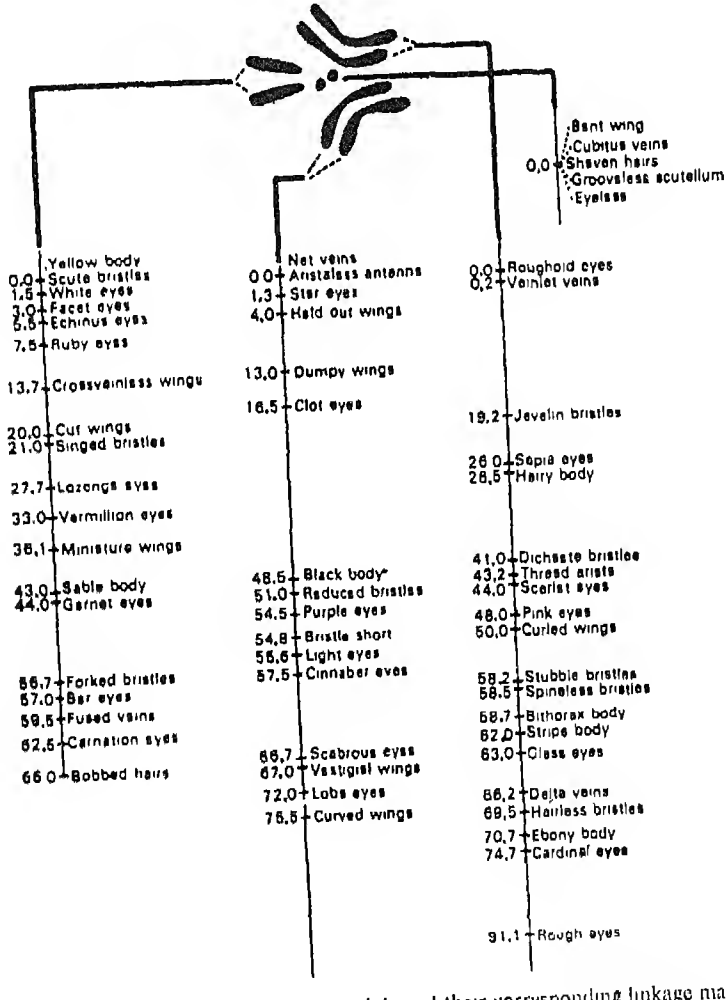


Fig. 18.5 Chromosomes of *Drosophila* and their corresponding linkage maps.

It occurred to Morgan and Sturtevant in the second decade of the present century that the relative distances between the genes on a chromosome can be estimated by determining the frequencies of recombinants. This information can be used to construct linkage maps of chromosomes, which can depict the order and relative distances

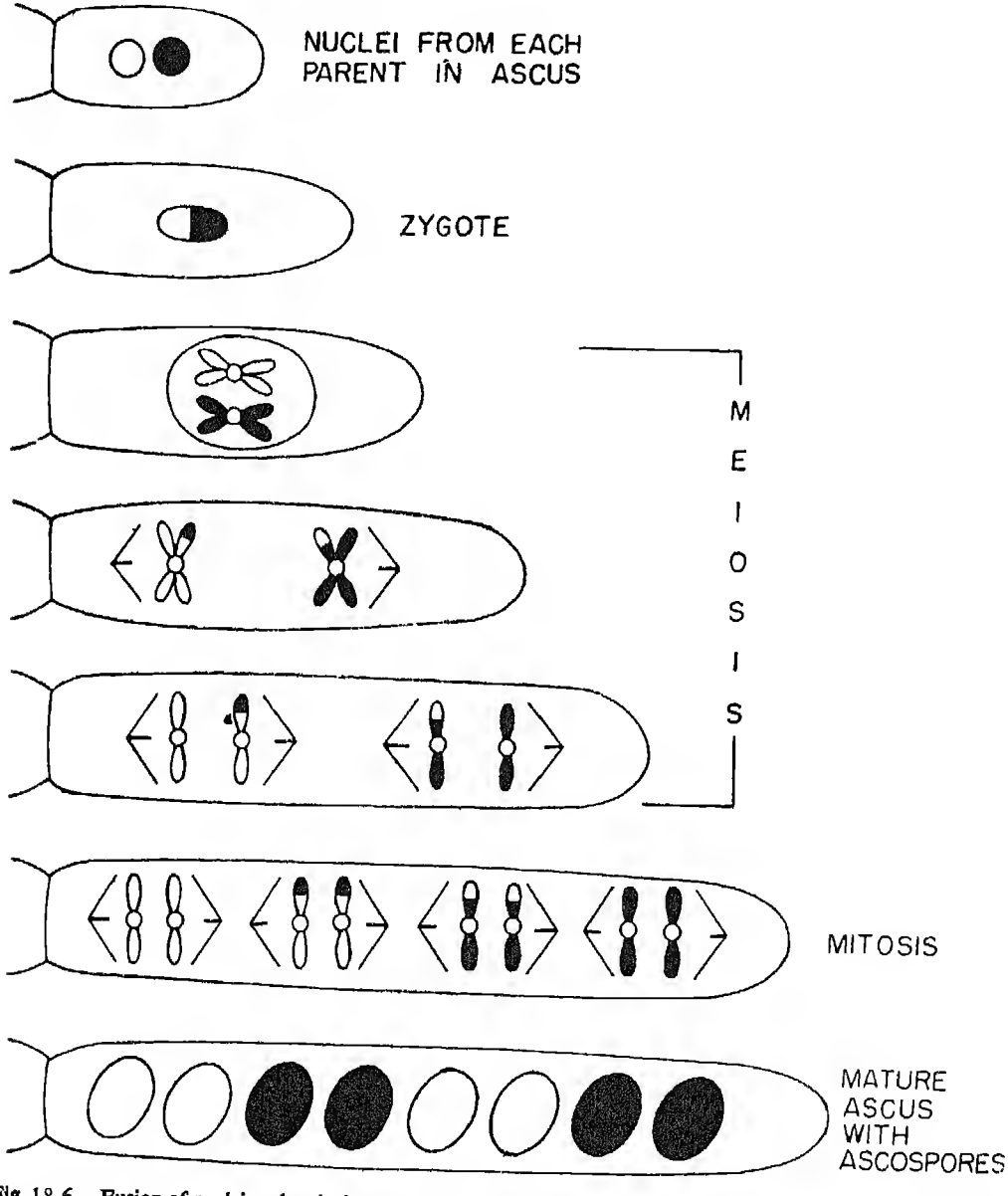


Fig. 18.6 Fusion of nuclei and meiotic and mitotic divisions in an ascus resulting in the 2 : 2 : 2 : 2 arrangement of ascospores.

between various genes. Linkage maps can be likened to linear road maps which indicate the relative distances between various places. It is interesting to note that linkage maps can be constructed, without looking at the genes and chromosomes, simply by making suitable crosses and by analysing the progeny characteristics with care and caution. Drosophila (Fig. 18.5) and maize were the first organisms in which linkage maps were constructed. Now, of course, the linkage maps of a variety of plants and animals are available. Even in human beings, a species in which controlled breeding experiments within a limited span of time are not possible, pedigree analysis and newer techniques of statistics and biochemistry are being increasingly used, with the result that respectable linkage maps are now available and details are forthcoming with great speed.

Crossing-over is the mechanism by which paternal and maternal genes and chromosomes are reassorted as a result of the exchange of parts of chromatids between homologous chromosomes. It occurs during prophase I of meiosis when the homologous chromosomes are paired to each other. Pairing starts during zygotene and is completed by the beginning of pachytene. During pachytene, it appears to consist of two chromatids. Thus, crossing-over occurs at a four-stranded stage. But at any given point only two of the four strands can be involved in crossing-over. As a result of

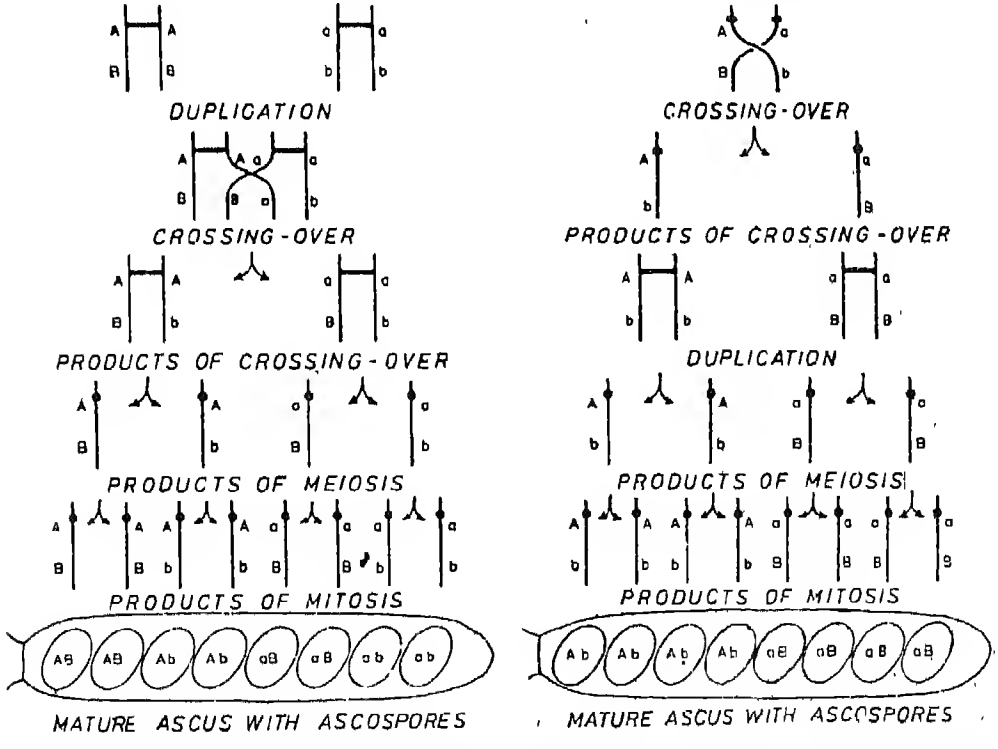


Fig. 18.7 Ascospore arrangements in *Neurospora* after crossing-over at a 4-strand (*left*) and a 2-strand (*right*) stage.

meiosis, four nuclei are formed, each containing one of the four chromatids. In the fungus *Neurospora*, the four products of meiosis remain linearly arranged one above the other. As a result of mitosis, each one of them gives rise to two nuclei. All the eight nuclei differentiate into ascospores which remain linearly arranged in a tubular ascus in the order in which they are produced. The ascospores are lined two by two (Fig. 18.6) because each pair has resulted from a mitotic division of a product of meiosis. By analysing the ascospores, the products of each meiosis and the orientations of chromatids during meiosis can be inferred. Neurospora is very suitable for this purpose because this is one of the few organisms in which all the products of meiosis are viable, and can be recovered and analysed. This point is well illustrated in Fig 18.7 The very fact that the two-by-two arrangements of ascospores are found in Neurospora shows that crossing-over occurs at a four-strand stage and only two of the four chromatids are involved in it at a given place. Had it been otherwise, there would have been only four-to-four arrangements *Neurospora* offers many advantages for genetical experiments because it can be cultured in defined media in the laboratory, it has a very short life cycle and its vegetative phase is haploid.

EXERCISES

1. What is linkage? What is its relationship with independent assortment and crossing-over?
2. Why was *Drosophila* chosen for most of the early experiments in genetics?
3. How was it proved that genes are located on chromosomes.
4. Draw the karyotypes of male and female *Drosophila* and highlight the differences between the two.
5. What is criss-cross inheritance? What is its importance?
6. What is a linkage group?
7. List the types of gametes produced from an individual of the genotype Ab/aB, with and without crossing-over between the two genes.
8. What is the relationship between (*a*) the physical distance between two genes, (*b*) linkage between them, and (*c*) crossing-over between them.
9. What is a linkage map? What is its basis? How can it be constructed?
10. Draw the stages of the prophase I, showing the stage at which crossing-over occurs and the consequences of it.
11. What is the evidence to prove that crossing-over occurs at the four-strand stage and not at the two-strand stage?

CHAPTER 19

Gene Expression and Interaction

GENES ARE linearly arranged on chromosomes. The hereditary material of chromosomes is a linear stretch of DNA or a sequence of bases. Genes, therefore, are nothing but a sequence of bases. Different genes have different base sequences Most of the genes are blue-prints for the synthesis of their complementary RNA. Some of this RNA is the structural component of organelles like ribosomes, some act as tRNA molecules (transport amino acids from the pool to the site of protein synthesis) and some are used as messengers for the synthesis of proteins. It is the last function of genes which was first recognised. While experimenting with the bread-mould *Neurospora crassa*, Beadle and Tatum found in 1948 that a heritable change in the structure of a gene can result in the absence of the activity of an enzyme. On the basis of their results with this fungus, they proposed the famous one gene - one - enzyme hypothesis which states that each gene is responsible for the synthesis of a specific protein or a particular enzyme. In recognition of their work, Beadle and Tatum were awarded a share of the 1958 Nobel Prize. Their contributions laid the foundations of biochemical genetics. Some recent studies on the structure of proteins have shown that some of the proteins may contain more than one polypeptide chain. Detailed genetic studies have shown that more than one gene may be responsible for the synthesis of a protein. Therefore, it is currently believed that one gene is responsible for the synthesis of one polypeptide chain or that the statement "one gene-one polypeptide chain" is nearer the truth. Some scientists prefer to use the term 'cistron' for the stretch of base sequences that codes for one polypeptide chain or mRNA transfer RNA (tRNA) or ribosomal RNA (rRNA) molecules which are responsible for synthesis of this chain. A cistron, therefore, can be defined as a functional unit of the chromosome.

Genes or cistrons which contain genetic information for tRNAs, rRNAs and proteins (some proteins catalyze certain reactions and are known as enzymes, whereas others form structural components of cell organelle) are known as structural genes. The activities of many structural genes are controlled by regulator genes through operator genes (see the chapter on enzymes).

Although details of gene expression have been worked out only recently, the fact that there is a close relationship between genes and enzymes was known just after the turn of this century. Archibald Garrod, a British doctor, discovered in 1909 that the inheritance

of alkaptonuria in man follows the pattern of a mendelian factor. Alkaptonurics have dark-coloured urine because of the presence of alkapton. Normal individuals possess an enzyme that catalyzes the oxidation of alkapton to carbon dioxide and water. Alkaptonurics, therefore, have a pair of recessive or defective genes. Mendelian inheritance and enzymic defects of a number of inborn errors of metabolism were clear in the early part of this century. But a direct relationship between genes and enzymes was established as a result of investigations on the fruit-fly. It was shown in the 1930s by many scientists that the synthesis of a normal red-eye pigment in *Drosophila* involves a series of enzyme-catalyzed reactions and that there is a gene for each of these enzymes. Beadle's and Tatum's expetiments with *Neurospora* were done against this background and led to the formulation of the one-gene-one-enzyme hypothesis. Thus, genes control the cellular function by synthesising the enzymes that catalyze the chemical reactions of the cell; these reactions, in turn, determine the phenotypic characteristics of an organism.

Mendelian principles can be explained on the basis of enzymic functions of genes. The dominant genes control the synthesis of active polypeptides whereas the recessive genes code for incomplete or defective (inactive) polypeptides. This is the reason why the dominant alleles are able to express a particular phenotype even in the presence of their recessive counterparts (for example, in a heterozygous condition). Thus, homozygous normal individuals have two genes for active alkapton oxidase, one on each homologous chromosome. Heterozygous individuals have one dominant gene which produces active alkapton oxidase, whereas the recessive gene produces an inactive form of this enzyme. Homozygous recessive patients have two defective genes, both of which control the formation of inactive enzymes.

In most of the cases, genes have all or none effect. Single genes are as effective as two of them. Consequently, heterozygous and homozygous individuals have similar phenotypes. There are many exceptions to this rule. Sometimes, genes have quantitative effects, e.g., in *Mirabilis jalapa*, colour of the flower. Homozygous recessive plants bear white flowers because the flower pigment is not produced. Heterozygous plants are able to synthesise only half the amount of the pigment that is produced by homozygous dominant plants. Consequently, they bear pink flowers — intermediate between red and white. In genetic jargon, the gene for red flowers in *Mirabilis* is incompletely dominant over its recessive allele. Such a system offers a distinct advantage to scientists. Just at a glance one can distinguish between a homozygous dominant and a heterozygous individual. Moreover, in such a case, the genotypic and phenotypic ratios in F_2 and subsequent generations remain the same (Fig. 19.1).

Many genes, like the gene for red flower colour in *Mirabilis*, are not essential for the survival of an organism. They do not control a vital function of the individual. As a result, the homozygous recessive individuals are viable. In some cases, such individuals are not viable and, therefore, the expected mendelian ratio is not obtained. The inheritance of sickle-cell anemia in human beings illustrates this point. This disease is caused by a gene with a lethal effect in the homozygous condition and only a slight but detectable effect in a heterozygous state. The red blood cells of the carriers of this disease assume sicklelike shapes under conditions of oxygen deficiency and occasionally show signs of mild anemia. The homozygotes

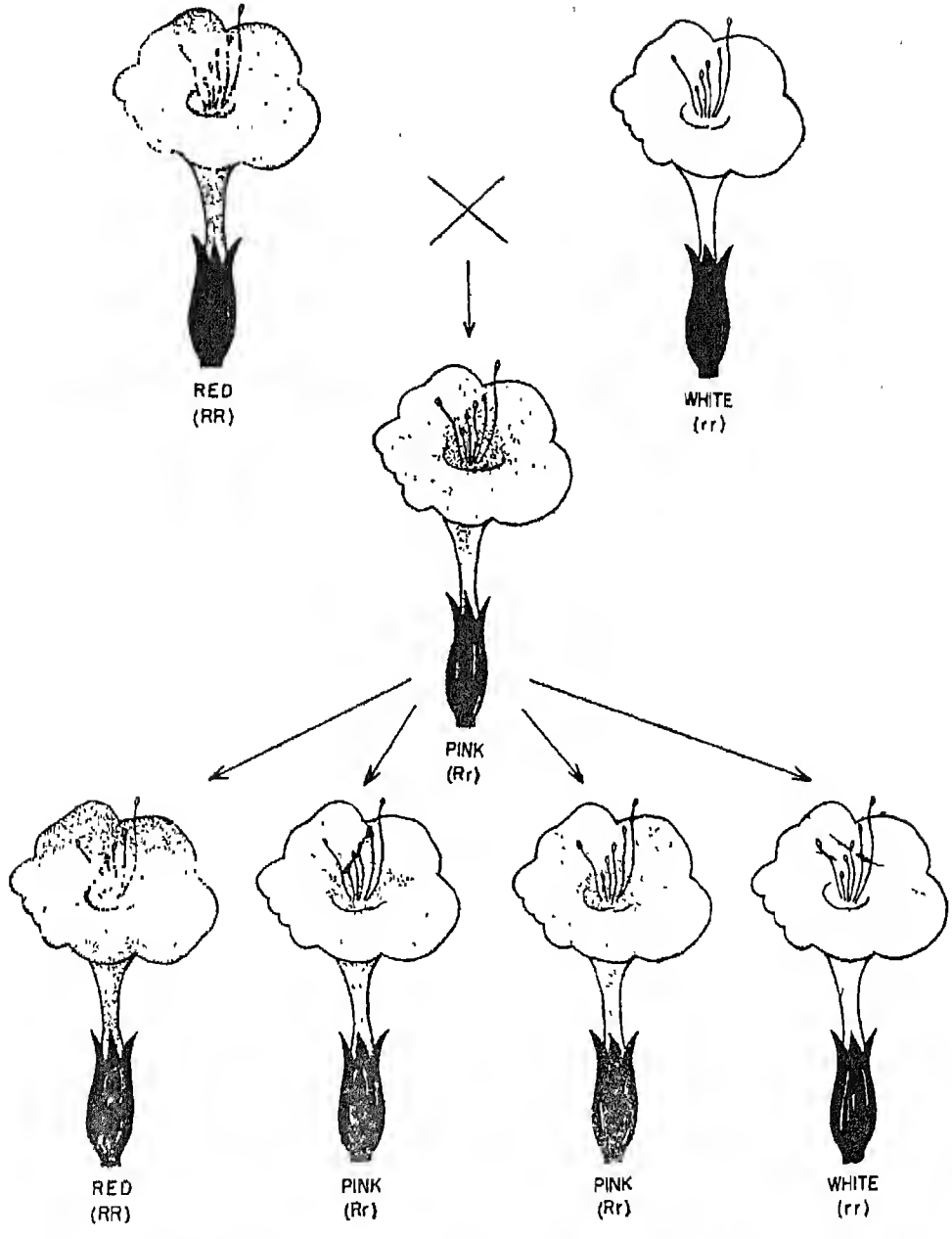


Fig. 19.1 Incomplete dominance in *Mirabilis* resulting in the same genotypic and phenotypic ratio in the F_2 generation.

generally die of fatal anemia before they attain sexual maturity. A marriage between two carriers, therefore, results in carrier- and disease-free children in a ratio of 2 : 1 (Fig. 20.2).

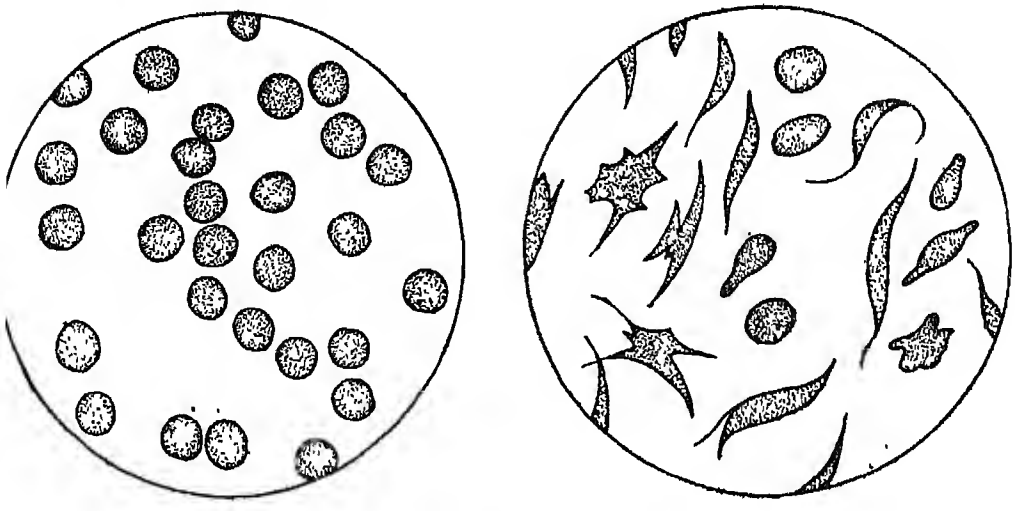
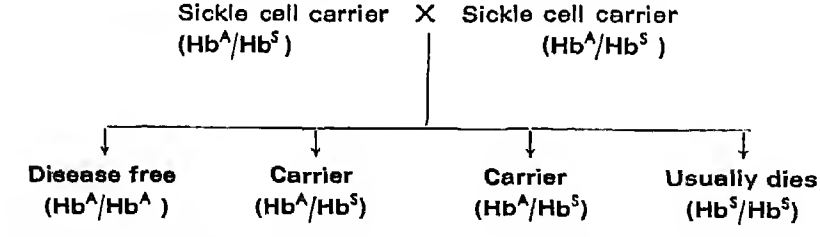


Fig. 19.2 Diagram of normal (*left*) and sickled (*right*) erythrocytes and the inheritance of sickle cell anemia in human beings (as shown below).



In some cases, the homozygous recessive individuals are normal and the homozygous dominant individuals die before maturity or just after birth. For example, matings between two mice with yellowish fur produce yellow and non-yellow in the ratio of 2 : 1. The zygotes homozygous for yellow are not viable (Fig. 19.3). Yellow body colour is dominant over black (non-yellow).

So far, we have discussed instances in which one character is controlled by one gene. There are examples in which more than one gene may affect the development and expression of only one character. In *Lathyrus odoratus* (sweet pea), the flowers are purple in the presence of two dominant genes — *C* and *P*. In the absence of both (*ccpp*) or either of the dominant genes (*cc PP*, *cc Pp*, *Cc pp* or *CC pp*), the flowers become white. The selfed progeny of heterozygous purple (*Cc Pp*) segregate into nine purple and seven white (Fig 19.4), instead of the mendelian dihybrid F_2 ratio of 9 : 3 : 3 : 1. The purple colour of the flowers of sweet peas, therefore, is the result of a complementary effect of the dominant alleles at two different loci, both of which segregate

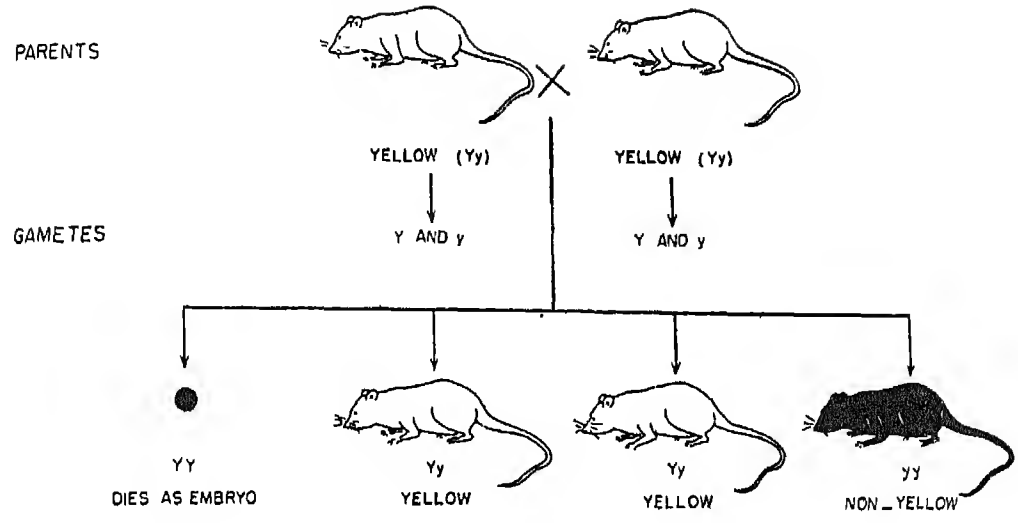


Fig 19 3 Inheritance of body colour in mice.

Parents: White CCpp × White ccPP

Gametes: Cp × cP

F_1: Purple CcPp

F_1 gametes →	CP	Cp	cP	cp
CP	CCPP Purple	CCPp Purple	CcPP Purple	CcPp Purple
Cp	CCPp Purple	CCpp White	CcPp Purple	Ccpp White
cP	CcPP Purple	CcPp Purple	ccPP White	ccPp White
cp	CcPp Purple	Ccpp White	ccPp White	ccpp White

F_2 Phenotypic ratio = 9 Purple : 7 White

Fig 19.4 Inheritance of flower colour in *Lathyrus odoratus*. Due to complementary genes, the F_2 phenotypic ratio of 9 : 7 is obtained.

independently of the other The extracts of white flowers appear colourless, but if the extracts from plants with different dominant genes are mixed together, purple colour develops. This indicates that the products of genes *C* and *P* are able to interact complementarily even in the test-tube. Alternatively, anthocyanin (the coloured pigment) is the product of two biochemical reactions, the end-product of one forming the substrate for the other

Gene 1 Gene 2

A ——— *B* ——— Anthocyanin

Evidences from studies on *Drosophila*, *Neurospora*, *E. coli* and a variety of other organisms have proved beyond all doubt that the expression of a particular character is the net result of a series of enzyme-mediated biochemical reactions, each one of which is gene-controlled. For example, the amino acid tryptophan in *E. coli* is synthesized from chorismic acid as a result of four sequential enzyme-catalyzed reactions which are shown with arrows in (Fig. 19.5. The numbers represent the genes which code for the various enzymes A defect in a particular gene acts as a barrier or block in the reaction sequence. A strain with any one or more of the four blocks is unable to synthesize tryptophan and requires it for growth. Thus, the phenotypic expression of a number of genes is the same in this example

In some cases, a single gene defect is expressed in a variety of characters, although

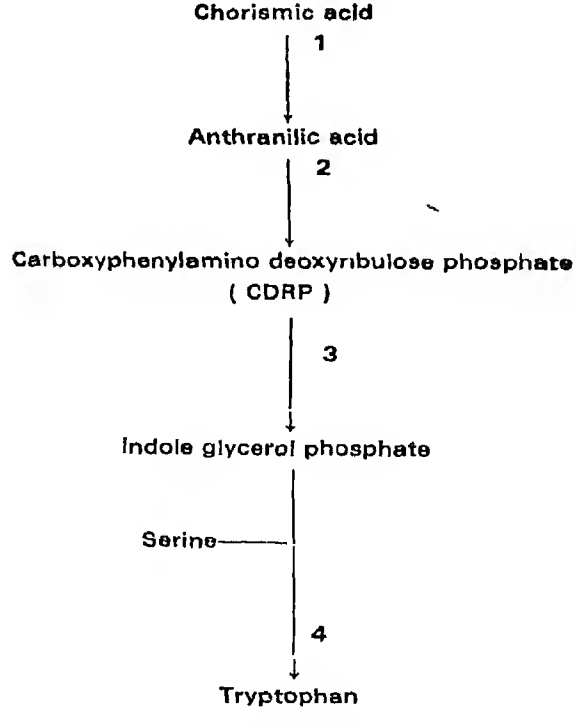


Fig. 19.5 Biosynthesis of tryptophan from chorismic acid in *E. coli*, involving four enzyme-catalyzed reactions (arrows) that are controlled by genes 1, 2, 3 and 4.

the primary effect is only one. For example, the gene for flower colour of sweet peas also controls the colour of seed coats and red spots in the axils of leaves. Such genes with multiple phenotypic effects are known as pleiotropic genes.

EXERCISES

1. Which of these statements are true and which are false?
   (*a*) Genes are composed of a linear sequence of nitrogenous bases.
   (*b*) Beadle and Tatum were awarded the Nobel prize for proposing the structure of DNA.
   (*c*) All genes are structural genes.
   (*d*) Sickle cell anemia has a lethal effect in a homozygous condition.
2. What is a cistron?
3. What are the contributions of *Drosophila* and *Neurospora* genetics to the one-gene-one-polypeptide chain hypothesis?
4. Why the dominant genes express themselves in the presence of their recessive alleles?
5. Do some genes have quantitative effects? Substantiate your statement with suitable examples.
6. "A lethal gene disturbs the expected phenotypic ratio." Justify this statement with a suitable example.
7. What are complementary genes? How are they inherited?

CHAPTER 20

Mutation

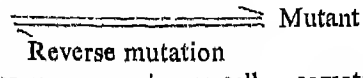
CHARLES DARWIN had postulated in his theory of evolution that the multiplication of individuals of a given species is accompanied with the origin of variation. Variation is essential for natural selection and struggle for existence. If all the individuals of a species in a population are alike, struggle for existence and natural selection cannot operate. Variations in a population arise as a result of two different mechanisms: (i) recombination and (ii) mutation. As has been discussed in Chapter 18, crossing-over results in new combinations of genes. This results in a population, different members of which possess different sets of characters. The sum total of characters in a population remains the same, but their permutations and combinations result in a diversity of genotypes and phenotypes. Mutation is another source of variation and, unlike recombination, results in the appearance of an altogether new character. Thus, mutation is the fountain-head of evolution.

The mutation theory was proposed by a Dutch scientist, Hugo de Vries. He was one of the three rediscoverers of mendelism.

Just after the turn of the present century, he noticed many heritable variations in the plant *Oenothera lamarckiana* and postulated (1901) that these changes are brought about by sudden and discrete changes in the germplasm of an organism. He also pointed out that these abrupt variations are important for evolution. Subsequent researches on a variety of organisms have shown that heritable changes can be brought about by changes in the structure of genes or by changes in the number or structure of chromosomes. Abrupt and distinct changes in the structure of genes are known as point mutations or simply mutations. Point mutations or gene mutations are detected because they bring about a perceptible change in the phenotype of the organism. Mutations that fail to bring about any phenotypic variation are lost undetected. It is only when a mutation occurs that we know that a particular character is controlled by a gene. Sometimes, the effect of a mutation is not very drastic and there is no perceptible change in the character. Nevertheless, such changes go on accumulating and play an important role in the evolution of a species.

A mutation generally results in the loss of a function. A change in the sequence of bases of a gene is reflected in an altered amino

acid sequence of the protein that it codes for. Such a changed protein may have reduced or no catalytic or functional activity. If an amino acid codon mutates to give rise to a nonsense codon, an incomplete polypeptide is synthesized. In both these cases, the normal function of the gene is lost. For example, the flowers of a normal pea plant are coloured because the plant is able to synthesize the pigment as a result of a series of biochemical reactions that are catalyzed by various enzymes. A mutation in any one of the genes that code for these enzymes will result in the absence of the flower pigment, thereby resulting in the production of colourless or white flowers. In a heterozygous condition, where the normal or the wild allele produces the pigment and the mutant allele is not capable of directing the synthesis of the pigment, each cell will possess the pigment. Such plants, therefore, will have coloured flowers. In other words, the mutant will be recessive to its wild type allele. Most of the mutants are recessive. In this example, the mutation of the wild type (coloured flowers) to white flowers is known as a forward mutation. A forward mutation, by definition, is a mutation from the wild type (original type) to a new type. A mutation may occur in the reverse direction too, i.e., from the mutant type to the wild type. Such a mutation is known as a reverse mutation. In the example given here, a reverse mutation will occur in the white-flowered plants to give rise to plants with coloured flowers.



Mutations may occur in any cell — somatic or reproductive. Mutations in reproductive cells are passed on to subsequent generations. If the induced mutation is recessive, it is not expressed until it becomes homozygous. Mutations in somatic cells are lost with the death of an organism, unless such cells are preserved due to vegetative propagation. Haploids are better for mutation studies because in them all the mutations, whether recessive or dominant, are expressed, as there is only one allele of each gene present in each cell. Many mutations are lethal because they result in the lack of a vital function. Such mutations can be preserved and studied under suitable conditions. For example, the bacterium *E. coli* can synthesize a whole variety of amino acids, vitamins, proteins, sugars and fats from the carbon and nitrogen sources and salts of a simple medium. This is possible as a result of the activities of various genes which code for the enzymes, proteins and RNAs required for the various metabolic processes. It is possible to isolate the mutants which have lost the ability to manufacture a particular kind of amino acid or a vitamin or any other essential organic compound. This happens because of a heritable change in the gene which codes for an enzyme which catalyzes one or more of the reactions leading to the synthesis of this organic compound. Such mutants are unable to grow in a simple medium but can grow if the compound which is not synthesized can be provided in the medium. Thus, a lethal mutation can be preserved under suitable conditions.

The mutant strains of micro-organisms and higher plants and animals, which have lost the ability to synthesize one or more essential compounds, are known as nutritional mutants or auxotrophs, in contrast to the original wild type which is known as the prototroph. Genetical and biochemical studies of auxotrophs and prototrophs have helped us a great deal in understanding the metabolic reactions and their control mechanisms in a variety of organisms. Nutritional mutants were first isolated in the bread mould *Neurospora crassa* by Beadle and Tatum in

Original codon sequence	CAT	CAT	CAT	CAT	CAT	.....
Codon sequence after the addition of a single base	CAA*	TCA	TCA	TCA	TCA	T.....

Fig. 20.1 A lateral shift in the coding frames due to the addition of a single base (A*). None of the codons remains in the same original position.

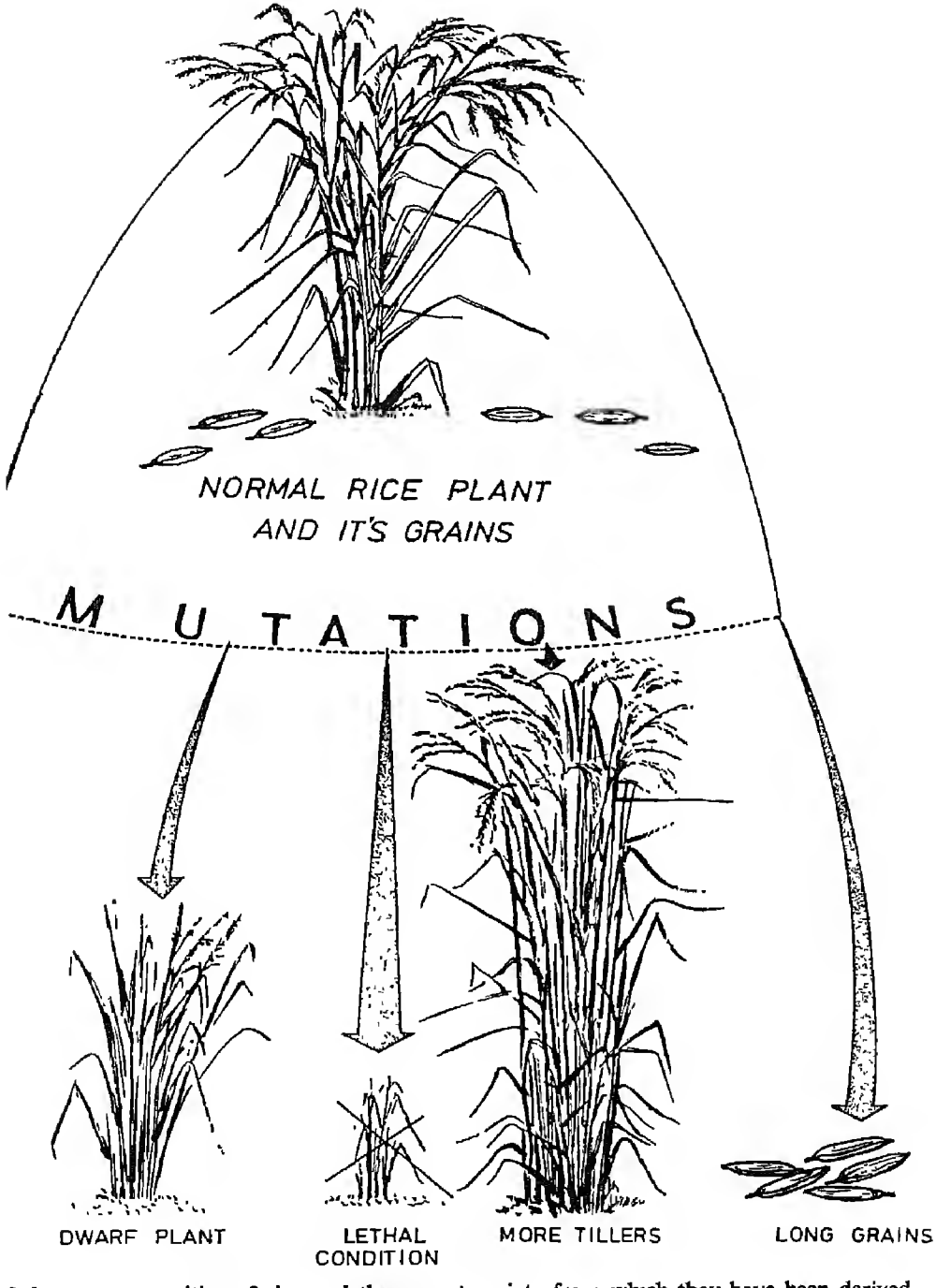
1944. They were the first to suggest a correlation between genes and enzymes.

Mutation is a random process and its frequency depends on the character and the organism studied. It is affected a great deal by the environmental conditions. The same organ or character may be affected by mutations in various genes. For example, in *Drosophila*, the red eye can change its colour due to a mutation in any of the *w*, *v*, *rb*, *br*, *car*, *lz* or *pr* genes. At the same time, a single mutation or a mutation in a single gene may affect a variety of characters. For example, a single mutation in *Pisum sativum* changes the seed coat colour from grey to white and the flower colour from red to white. Mutations which have more than one phenotypic effect are known as pleiotropic mutations.

Spontaneous mutation frequencies are very low. Methods are now known to increase the rate of mutation by artificial means. Agents which increase the rates of mutation are known as mutagens. Early attempts at increasing mutation frequencies by artificial methods were greatly handicapped by the absence of suitable techniques of identifying and measuring mutation rates. As soon as these techniques were available, a number of mutagens were discovered. The first success came to Muller in 1927, who showed that the exposure of *Drosophila* to X-rays increases the mutation rate of some characters by about 150-fold He also noticed that within a certain range, increasing X-ray doses increased the mutation frequencies in *Drosophila*. The same was found to be true for barley (Stadler, 1928) and, subsequently, for a variety of other organisms All forms of energy that disrupt the chemical structure of chromosomes, e.g. ultra-violet light, X-rays, gamma rays, beta rays, cosmic rays, etc., have been found to be mutagenic in almost all the organisms. This is the reason why there is increasing public concern about the human activities that increase the background levels of environmental radiation Mutant cells can also be created through genetic surgery. In this process, the cells are grown in culture until they reach the late prophase or metaphase stage when the chromosomes are most visible and then a fine laser beam is used to disrupt and delete selected parts of chromosomes.

A variety of chemicals have also been found to act as mutagens. Some of them react with the bases of DNA and convert them into unusual or abnormal bases (nitrous acid deaminates cytosine to uracil), thereby changing the code word. Some other chemicals resemble normal DNA bases (base analogoues like 5-Bromouracil) in structure and, therefore, get incorporated by mistake in the DNA chain, in place of the usual base, during DNA synthesis. This insertion causes mistakes during replication and ultimately leads to a heritable mutation in the gene. The third group of compounds (acridines) gets inserted in between two bases of a DNA chain and ultimately result in the addition or deletion of a few bases. This results in a lateral shift of the coding frames, thereby changing the whole lot of codons (Fig. 20.1). Such mutations are known as gibberish or frameshift because they result in a sequence of codons that code for a polypeptide chain which make no sense

Some of the bases of a nucleotide chain are more prone to mutation than others. At the same time, some sites mutate much more frequently due to certain mutagens than due



f the mutant varities of rice and the parent variety from which they have been derived

to others. Mutagen specificity occurs for organisms, too.

Mutations are useful for evolution of the species as well as for understanding the basic principles of inheritance and cell metabolism. They are also of immediate use to mankind. Mutant varieties of wheat, which are dwarf, early maturing, resistant to various diseases and have better and more protein content, have gone a long way in ameliorating the miseries of mankind (Fig. 20.2). Mutant varieties of rice (Fig. 20.2) which are dwarf or which have many more tillers or long grains, are very popular with the farmers. Mutants with curved grains or lethal condition are useful for research workers. Almost all the varieties of crops that are grown today are spontaneous or induced mutants with better yield and have been derived from the pre-existing varieties. Various mutant strains of micro-organisms with better fermenting capabilities or with better yields of antibiotics or other useful compounds have been isolated and are being used in various industries. The same is true of pets and cattle. The Ancon breed of sheep with very short legs arose as a single germinal mutation from the normal variety. It is because of the usefulness of some mutants that a large number of research centres are engaged in the production and selection of mutant varieties of plants and animals. At the Agricultural Research Institute in New Delhi, plants are grown in a large field which is shielded by a high wall and which has a source of gamma rays in the centre. After being exposed to the desired dose of gamma rays for the desired period of time, the plants are taken out and their progeny analyzed for induced useful mutations.

Once a suitable mutation is induced and detected in an organism, it is multiplied and transferred to desired individuals by controlled breeding experiments. Crosses between males and females are the conventional ways of transferring a character from one individual to the other. More recently, some novel ways have been developed to achieve this. The prospects and limitations of these techniques of genetic engineering have been dealt with in the last chapter of this section. All these techniques facilitate the best use of a mutant, once it is induced and selected.

During recent years, there has been an ever-increasing social concern about our ability to produce mutants and to manipulate the genotype of an organism. Some countries have gone so far as to impose legal restrictions on some kinds of genetic manipulations.

EXERCISES

1. What are the sources of variation in a population?
2. Why a mutation generally results in the loss of a function?
3. Haploids are more suitable than diploids for mutation work. Why?
4. Write explanatory notes on the following:
   (*a*) Auxotroph
   (*b*) Prototroph
   (*c*) Mutagen
   (*d*) Reverse mutation
   (*e*) Pleiotropic mutation
5. How can the frequencies of mutations be increased?
6. Discuss the modes of actions of at least two mutagens.
7. Are some mutations useful? Give specific examples.
8. What are frameshift mutations? What is the effect of such mutations?
9. Can a lethal mutation be preserved?
10. Do all mutations that occur spontaneously get preserved in nature or do some of them get lost forever?

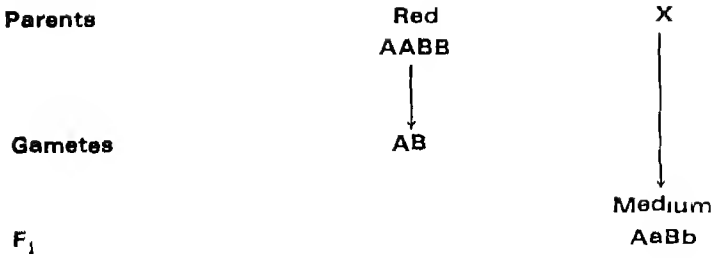
CHAPTER 21

Quantitative Inheritance

IT WAS mentioned in the previous chapter that mutations result in abrupt changes or discontinuous variations in the phenotype. Continuous variation occurs due to environmental variation. Generally speaking, this is true. But there are exceptions to the rule. It was F. Galton who suggested way back in 1883 that many instances of continuous variation are heritable rather than environmental. He was impressed by the fact that taller human beings produce taller children on the average. He suggested that characters like height and mental capabilities in human beings are heritable, although they show a continuous range of variation in a population. Galton's postulate gained experimental support when it was found that at least in some instances the same character can be determined by more than one gene, each with the same but cumulative phenotypic effect. Quite a few quantitative characters like plant height, yield of crops (size, shape and number of seeds and fruits per plant), intelligence in human beings, milk yield in animals, etc., have been found to be determined by many genes and their effects have been found to be cumulative. Each gene has a certain amount of effect, and the more the number of dominant genes, the more accentuated is the character. Quantitative inheritance is also known as polygenic inheritance or multiple factor inheritance.

Experimental evidence for polygenic inheritance was first obtained by a Swedish geneticist, H. Nilsson-Ehle, in 1908. He found that the kernel colour in wheat is determined by three gene pairs: *Aa*, *Bb* and *Cc*. Genes *A*, *B* and *C* determine the red colour of the kernel and are dominant over their recessive alleles *a*, *b* and *c* which result in white kernels Each gene pair shows mendelian segregation. Thus, heterozygotes for one gene pair (*Aa bb cc*, *aa Bb cc* or *aa bb Cc*) segregate into three red and one white kerneled plant. Heterozygotes for two genes (*Aa Bb cc*, *Aa BB Cc* or *aa Bb Cc*) segregate into 15 red and one white kerneled plant, whereas heterozygotes for three genes (*Aa Bb Cc*) segregate into 63 red and one white kerneled plant. But all the red kernels do not exhibit the same shade of red Different genotypes show different degrees of redness, the intensity depending on the number of the dominant genes present (Fig. 21.1).

Another common example of quantitative inheritance is the inheritance of skin colour



F_1 gametes →	AB	Ab	aB	ab
AB	AABB Red	AABb Dark	AaBB Dark	AaBb Medium
Ab	AABb Dark	AAbb Medium	AaBb Medium	Aabb Light
aB	AaBB Dark	AaBb Medium	aaBB Medium	aaBb Light
ab	AaBb Medium	Aabb Light	aaBb Light	aabb White

(F_2)

Summary of $F_2 = \frac{1}{16}$ Red : $\frac{4}{16}$ Dark : $\frac{6}{16}$ Medium : $\frac{4}{16}$ Light : $\frac{1}{16}$ White

Fig. 21.1 Results of a cross between a wheat variety with red kernels (homozygous for two dominant genes) and another variety with white kernels. Different genotypes show different degrees of redness

in man. Melanin is the pigment which determines the colour of the skin. The more the pigment, the darker is the skin An analysis of the intensity of the pigments in individuals of different races, their hybrids and subsequent generation progeny has revealed the polygenic nature of inheritance of the skin colour. The F_1 progeny of a cross between a white and a negro individual shows an intermediate colour of the skin In the second generation, the range of colour variation is more as is evident from the analysis of melanin percentage in 16 progeny of the second generation of white and negro parents (Table 21 1) On the basis of these results, Davenport (1913) proposed that skin pigmentation is determined by at least two pairs of alleles and that each dominant gene is responsible for the synthesis of a fixed amount of melanin. The effect of all the genes is additive and the amount of melanin produced is always proportional to the number of dominant genes. Subsequent studies have shown that as many as six genes may be involved in controlling the colour of skin in human beings. The pattern of inheritance of the skin colour in human beings is not easy to analyse because it

TABLE 21 1

Genotypes and Phenotypes of the Second Generation Progeny of White (*aa bb*) and Negro (*AA BB*) Parents

Genotype	*Frequency*	*Phenotype*	*Phenotypic ratio*	*Percentage of melanin*
AA BB	1	Black	1	56—78
Aa BB	2	Dark	4	41—55
AA Bb	2			
Aa Bb	4	Intermediate	6	26—40
aa BB	1			
AA bb	1			
Aa bb	2	Light	4	12—25
aa Bb	2			
aa bb	1	White	1	0—11

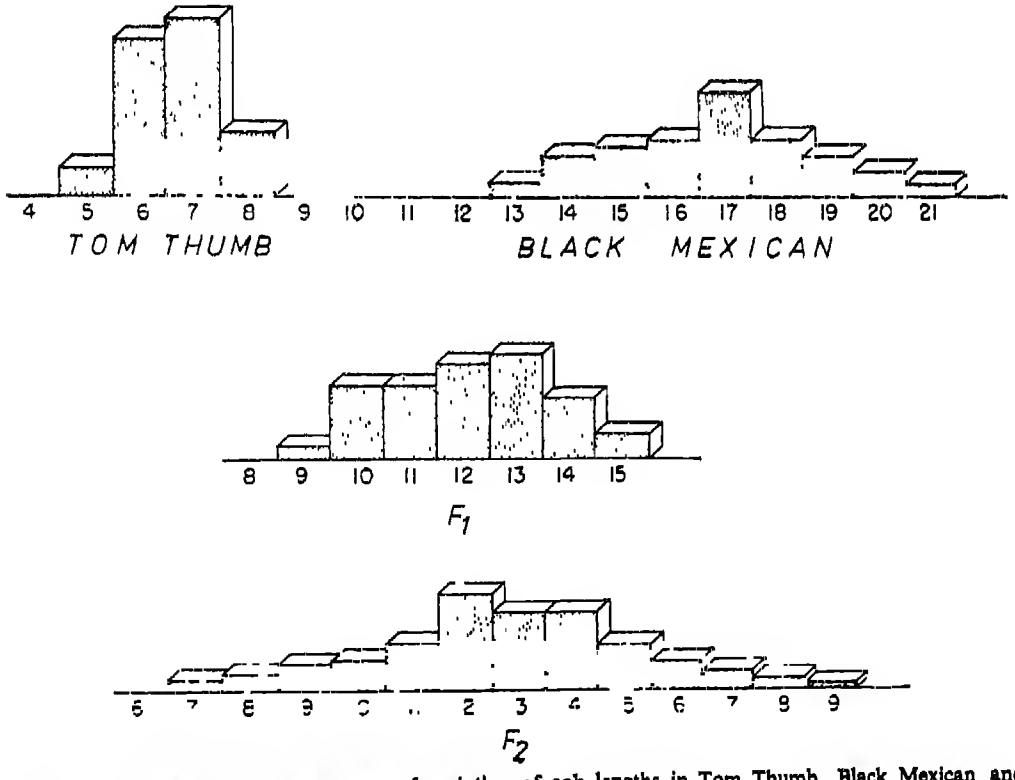


Fig. 21.2 **Histograms showing ranges of variation of cob lengths in Tom Thumb, Black Mexican and their F_1 and F_2 progeny.**

changes under the influence of age, cosmetics and environment and the number of progeny of each marriage is finite

The pattern of polygenic inheritance is easier to analyse in plants because, in them, controlled matings can be performed and because each cross results in a large number of progeny which can be subjected to statistical analyses. One of the earliest and most thoroughly studied cases of quantitative inheritance in plants is the inheritance of cob length in maize. Emerson and East (1913) crossed the Tom Thumb variety with the black Mexican variety. The cobs of Tom Thumb were 5 to 8 cm (average=6.6 cm) long, whereas the cobs of the black Mexican variety were 13 to 21 cm (average=16.8 cm). Emerson and East found that the F_1 progeny had cobs of intermediate length, ranging from 9 to 15 cm (average=12.1 cm). But in F_2, there was a wider range of variation, 7 to 19 cm, the average lying close to the F_1. The extreme phenotypes extended into the respective ranges of the two parental strains (Fig. 21.2). On the basis of these results, it has been proposed that two gene pairs are involved in determining the length of cobs in maize. In the absence of the dominant genes, the cob length is 6.6 cm, as in the Tom Thumb variety Each dominant gene has the same effect on the cob length and adds to the basic length (6.6 cm) of the cob.

$$\frac{16\,8 \text{ (average of Black Mexican)} - 6.6 \text{ (average of Tom Thumb)}}{4 \text{ (number of genes for cob length)}} = 2.55 \text{ cm (contribution of each gene)}$$

This is evident from Table 21.2 which gives the cob lengths of different genotypes of F_2 progeny of a cross between the Tom Thumb (*aa bb*) and the black Mexican (*AA BB*) variety.

TABLE 21 2

Average Cob Lengths of Different Genotypes of the F_2 Progeny of a Cross between the Tom Thumb and the Black Mexican Varieties of Maize

Genotype	*Relative frequency*	*Cob length in cm*	*Phenotypic ratio*
AA BB	1	16.8	1
Aa BB	2	14 2	
AA Bb	2	14.2	4
Aa Bb	4	11.7	
aa BB	1	11.7	6
AA bb	1	11.7	
aa Bb	2	9.1	4
Aa bb	2	9.1	
aa bb	1	6.6	1

In all cases of polygenic inheritance, extreme phenotypes are rare and the intermediate ones are more frequent. As the number of segregating alleles increases, the chances of getting F_2 progeny similar to either parent decreases and the number of intermediate classes increases.

If the results of polygenic inheritance are plotted as a histogram to show the distribution patterns of various classes, in subsequent generations the difference from the pattern of a mendelian segregation becomes very

obvious. In a typical monohybrid mendelian segregation, the two parents fall in two distinct phenotypic classes — homozygous dominant and homozygous recessive. In F_1, due to the dominance of one allele over the other, all the progeny show one of the parental characters. The second generation shows segregation of the dominant and the recessive phenotype in the ratio of 3 : 1. On the other hand, in the case of a polygenic inheritance the parents fall into two distinct classes, but the F_1 shows an intermediate character because of the dilution of the dominant genes. The F_2 shows a still wider spread. A diagrammatic comparison of a monohybrid mendelian segregation (monogenic) and a polygenic inheritance is shown in Fig. 21.3.

A comparison of the histograms showing the phenotypic distribution of F_2 of one, two and three gene segregations (Fig 21.4) shows that the greater the number of segregating genes, the wider is the spread. Thus, from the nature of frequency distribution one can have some idea about the number of genes involved in polygenic inheritance.

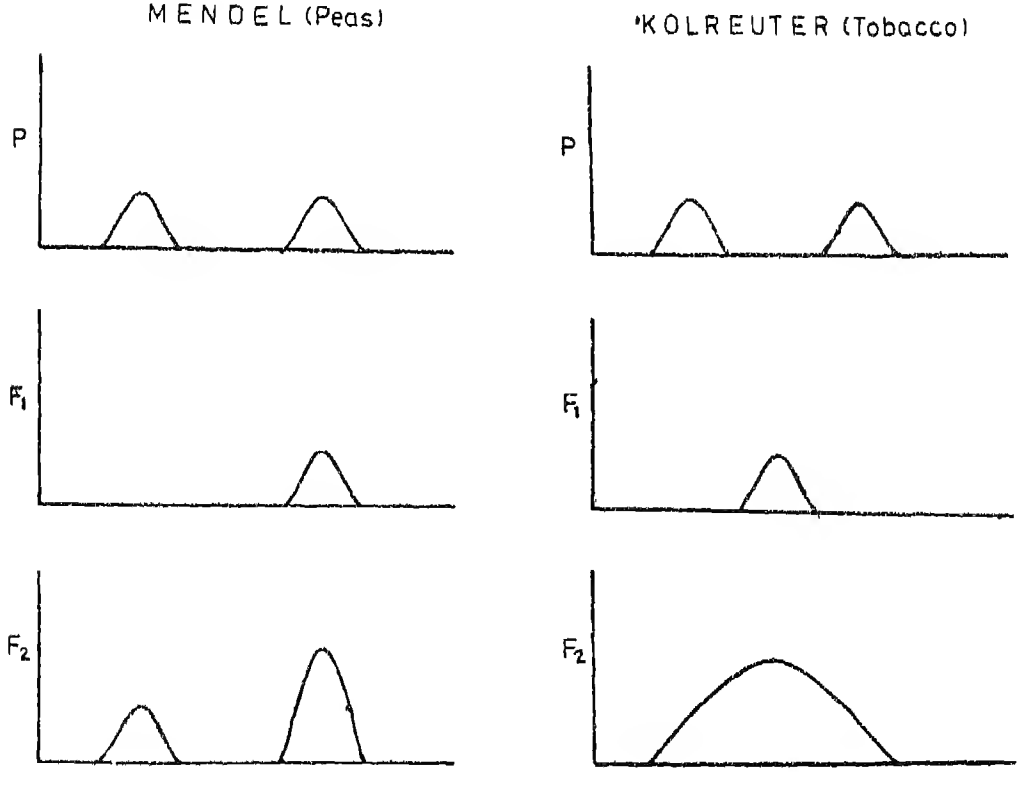
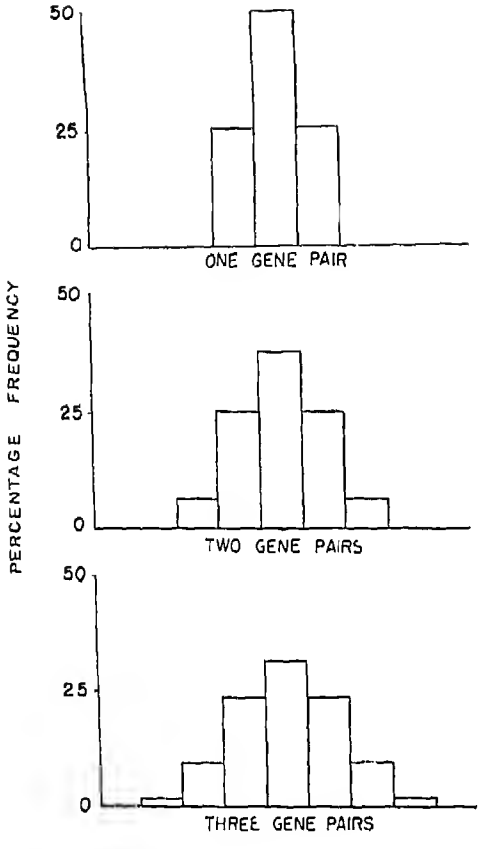


Fig. 21.3 Graphic comparison of the results of monogenic (*left*) and polygenic (*right*) inheritance. The top row shows the phenotypic distribution of parents, whereas the middle and bottom rows show the phenotypic distribution of F_1 and F_2 progeny, respectively.



Many examples of polygenic inheritance are known in plants and animals. Most of them are concerned with easily recognisable quantitative characters. It is generally believed that during evolution there was duplication of chromosomes or chromosome parts, thereby leading to multiple copies of the same gene.

Some of the quantitative characters are controlled by single genes as well as by more than one gene in an additive or cumulative fashion. For example, the tall character in sweet pea is controlled by polygenes as well as by a single gene pair. There is a range of variation between tall and dwarf plants due to different numbers of dominant genes in different phenotypes. At the same time, a single mutation in the tall plant may result in a dwarf individual.

Fig 21.4 Histograms showing the phenotypic distribution of F_2 with one, two and three segregating gene pairs.

EXERCISES

1. What led Galton to suggest that some of the heritable variations are continuous rather than discontinuous?
2. Discuss an example of polygenic inheritance
3. Describe the pattern of inheritance of the skin colour in mice.
4. Why is it easier to analyze the pattern of inheritance in plants than in animals?
5. Highlight the differences between the patterns of monogenic and polygenic inheritance.
6. How can the nature of frequency distribution give some idea about the number of genes involved in polygenic inheritance?

CHAPTER 22

Human Genetics

MENDEL'S principles of inheritance are applicable to all living organisms. Man is no exception Other basic principles of genetics, as discovered by experiments with bacteria, fungi, fruit-fly, maize, etc, are equally applicable to human beings. During early years of genetics, man was not a suitable organism for studies on inheritance because he is not amenable to controlled breeding experiments, the number of progeny of each marriage is small and the life cycle is too long Most of the early genetical investigations, therefore, depended on the pedigree analysis. But during recent years, newer techniques have been developed and we have been able to understand a lot about the mode of inheritance of a large number of characters in human beings. The five basic approaches to human genetics are as follow:

(1) Pedigree records these days are well-recorded and well-maintained. This helps in knowing whether a particular character is inherited or not. It is also easy to trace the transmission of a particular trait through generations.

(2) The limitations imposed by the small number of progeny produced (brood size) have been overcome by the methods which can analyse the fate of characters in populations Population genetics has emerged as a very useful and productive field of biology during recent years and has been used extensively in the study of human genetics.

(3) The advent of biochemical genetics and the techniques to culture human cells in defined media in flasks, tubes and petri dishes, coupled with the techniques of somatic cell genetics, have helped a lot in understanding the biochemical bases of inheritance of a number of traits. They have also helped in understanding the physiology of growth and development in human beings.

(4) Human cytology has been made much easier. Until the year 1956, the correct number of somatic chromosomes in man was not known. But now it is possible to place man and mouse chromosomes in the same cell and it is also possible to selectively eliminate some of the chromosomes from such a somatic hybrid.

(5) The hereditary basis of quite a few characters has been established by comparing the 'phenotypes of identical and fraternal twins. Identical twins come from a common zygote, by the separation of two-celled embryo into two independent cells, each of

which develops to form separate individuals. Identical twins, therefore, have similar genetic constitutions, except for rare chance mutations. Fraternal twins, on the other hand, arise from different fertilizations that have occurred at the same time. In other words, fraternal twins come from a double ovulation. Such twins, therefore, are no more similar than any other brothers and sisters, and generally show similarities of at the most only 50 per cent of the characters. Thus, the characters which show dissimilarities in identical twins are environmental, rather than heritable. The degree of heritability in identical and fraternal twins can be analysed by statistical methods.

Human genetics has advanced so much that people are talking about cloning human beings and tailoring the phenotype by genetic engineering. Human genetics had its birth in 1901 when Sir Archibald Garrod, a British physician, pointed out that inborn errors of

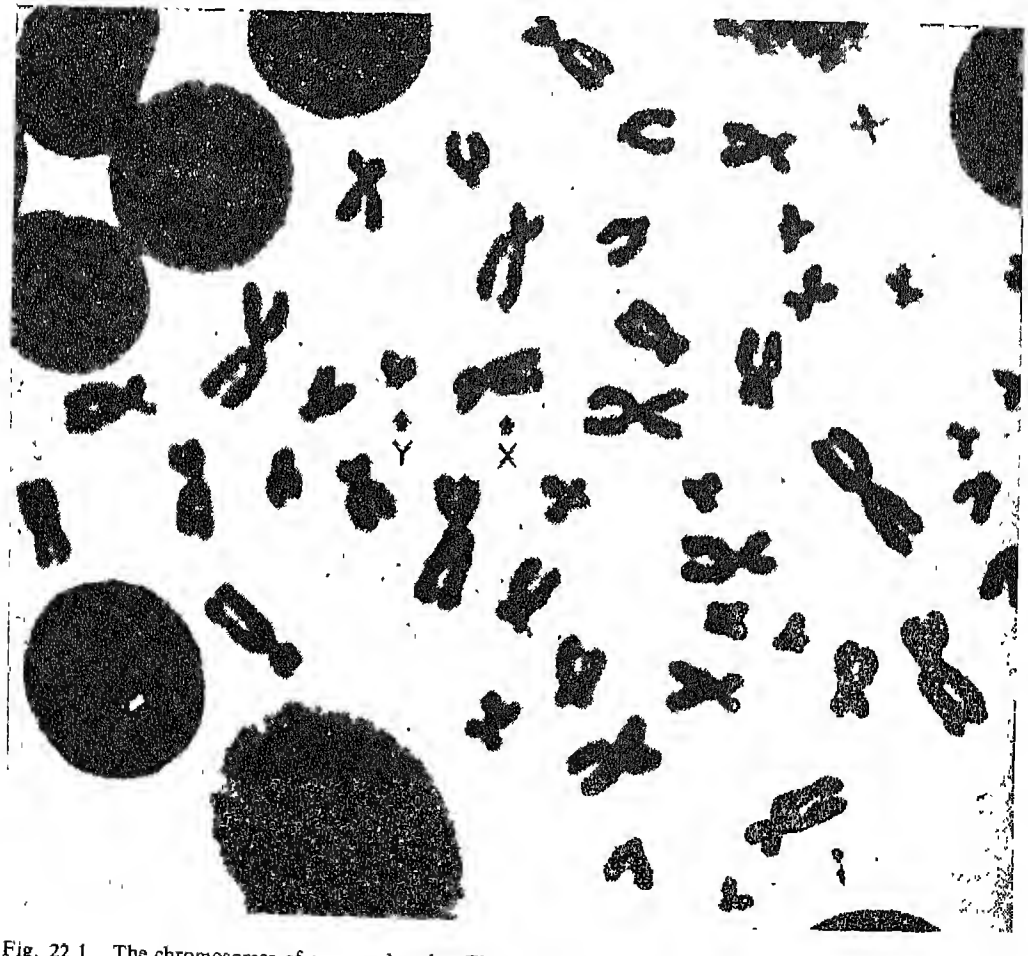


Fig. 22.1 The chromosomes of a normal male The X- and Y-chromosomes are marked with arrows.

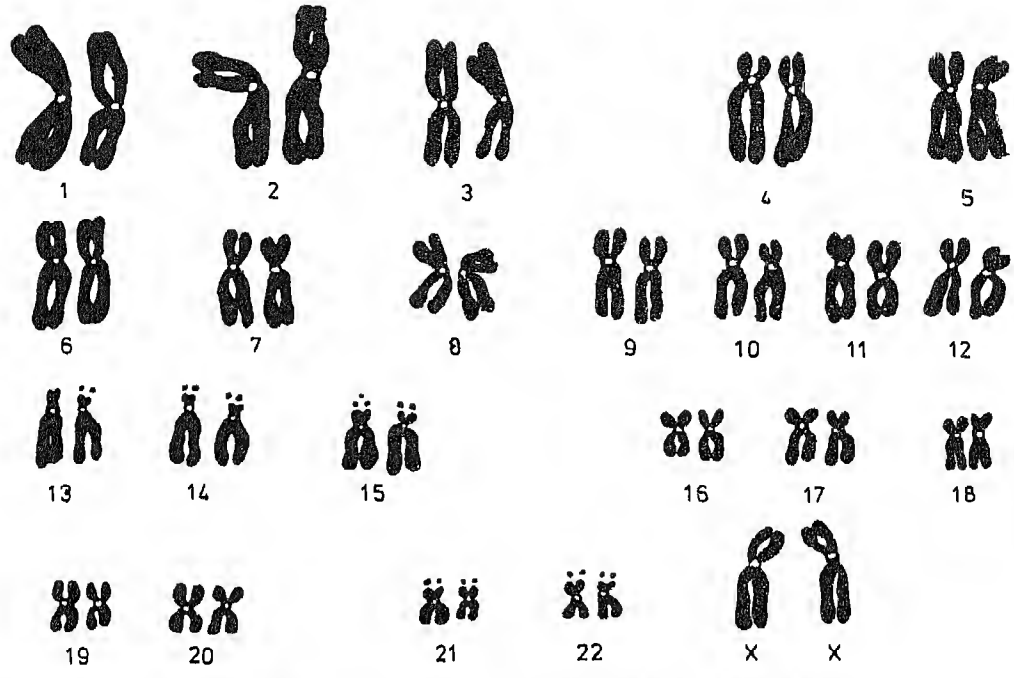


Fig. 22.2 The chromosomes of a normal female arranged in pairs.

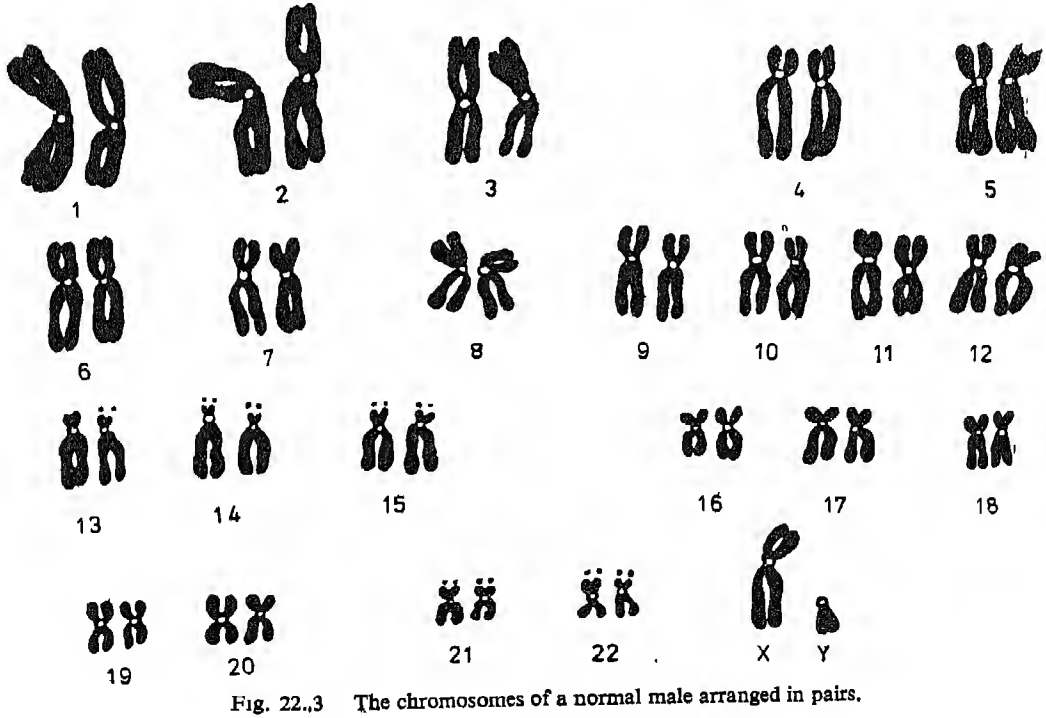


Fig. 22.3 The chromosomes of a normal male arranged in pairs.

metabolism are gene-controlled and are inherited in a mendelian fashion. Since then, a large number of deformities, malformations and diseases have been shown to be inherited. Some of them have been shown to be gene-controlled, whereas others have been found to be associated with gross chromosomal abnormalities.

Human Chromosomes

The number of somatic chromosomes in man was found to be 2n=46 by Tjio and Levan in 1956. Thus, each cell of our body contains 23 pairs of chromosomes (Fig. 22.1). Of these 23 pairs, 22 pairs are similar in males and females. These are called autosomes. Both the chromosomes of the twenty-third pair are similar in females (Fig. 22.2) but dissimilar in males (Fig. 22 3). In males, one of the chromosomes of this pair is long and similar to the twenty-third pair of chromosomes of females, but its partner is small In both the sexes, the twenty-third pair is known as the sex chromosome pair. The female sex chromosomes are known as XX and the male as XY. Each chromosome pair has a distinct morphology, as regards the relative lengths of its two arms and the position of the centromere. The chromosomes can be artificially arranged by selecting the photographs of homologous chromosomes and by putting them side by side, different pairs arranged in the order of their lengths, except the sex chromosomes which are placed at the end. This arrangement shows the karyotype or the relative morphologies of chromosomes very clearly. By this technique all the 23 pairs of human chromosomes have been properly identified and numbered. Any gross morphological change in any of the chromosomes can be easily detected. During recent years (since 1969), techniques have been developed to stain the human chromosomes with fluorescent dyes after various treatments. Different treatments result in different banding patterns (alternate bands of stained and unstained regions) along the length of a chromosome. The banding pattern of a particular chromosome remains constant for a particular treatment and each chromosome shows a unique

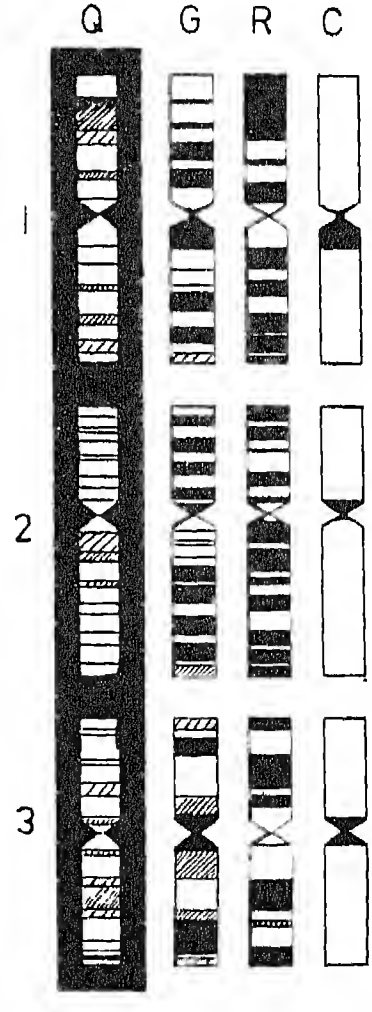


Fig. 22 4 Quinacrine (*Q*), giemse (*G*), reverse of giemsa (*R*) and constitutive heterochromatin (*C*) bands of the three largest chromosomes of human beings.

pattern with the same treatment. This technique helps in identifying the various regions of individual chromosomes. At present, four different types of banding patterns are known, and they are known as Q, G, R, and C patterns according to the treatments to which the chromosomes are subjected (Fig. 22.4) Cytological studies of human chromosomes have helped a lot in correlating various congenital malformations with abnormalities of chromosome number and structure. It has been estimated that chromosomal abnormalities are present in about four to five out of every 1000 live births and in one out of every five spontaneous abortions. The chromosomal abnormalities may involve autosomes or sex chromosomes.

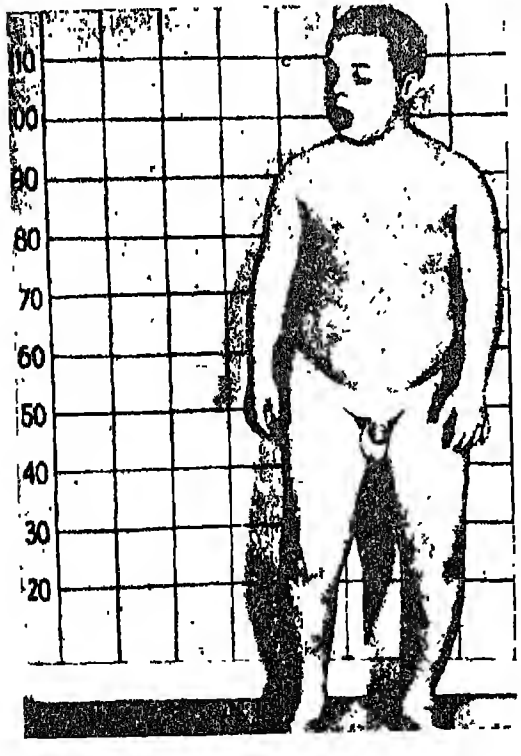


Fig. 22.5 An individual showing Down's syndrome.

Autosomal Abnormalities

Mongolism or Down's syndrome (Fig. 22.5) was first reported in 1866. The affected children have a very broad forehead, short neck, flat hands, stubby fingers, permanently opened mouth, projecting lower lip and a long extending tongue. The victim suffers from a malformation of the brain and, thus, has little intelligence. The deformities are also often found in the heart and other organs Although this disease was known for quite some time, its real cause was discovered only in 1959. It was found that the patients possess 47 chromosomes instead of 46, the twenty-first chromosome being present in three doses. This small extra chromosome carries enough excess genetic material to disrupt the normal development and phenotype Down's syndrome is a very common congenital abnormality and occurs almost once in every 600 births. It is now known that this extra chromosome comes due to an error during the formation of the egg cell. The chromosomal studies have revealed that the mistake is due to the failure of separation of the twenty-first pair of chromosomes during meiosis Thus, an egg is produced with 24 chromosomes, instead of 23 Such defects occur mostly in the ovaries of aged women. This is the reason why such abnormal children are born to mothers who are above 40 years of age

Like Mongolism, several other kinds of disorders have been reported, which are due to alternations in the chromosome complement. Most such frequent alternations in the chromosome complement are due to an extra eighteenth (Edward s syndrome), first (Patan's syndrome) and eighth, ninth or thirteenth chromosome. The absence of one of the twentieth chromosome pair, so that each cell has only one chromosome of this type, also results in congenital malformations. Sometimes, only parts of chromosomes

are missing or are in excess in mentally retarded or phenotypically abnormal cases. For example, an excess of a part of the thirteenth or fifteenth chromosome has been found to be associated with various phenotypic aberrations Exchange of non-homologous segments between two chromosomes and inversions or deletions of parts of some chromosomes also result in various malformations and reduced fertility.

Sex Chromosomal Abnormalities

Numerical aberrations involving sex chromosomes are much more frequent than the aberrations involving autosomes. Many well-known syndromes have been found to be associated with changes in the number of sex chromosomes, which can be classified into the following four types:

(*a*) Presence of only one X-chromosome in females or Turner's syndrome (XO). Such females are characterized by short stature, retarded sexual development, sterility, webbing or looseness of the skin of the neck and other abnormalities. It occurs with a frequency of one in every 3000 new births.

(*b*) Presence of extra X-chromosomes in males or Klinefelter's syndrome (XXY, XXXY, XXXXY, XXYY, XXXYY, etc.). Such males are characterized by feminized secondary sexual characters, long limbs, sterility, degeneration of seminiferous tubules, limited intelligence and mental retardation. The greater the number of X-chromosomes, the severer is the mental defect.

(*c*) Presence of extra X-chromosomes in females (XXX, XXXX or XXXXX), thus resulting in 47, 48 or 49 chromosomes in each cell. Such females show abnormal sexual development and mental retardation. The symptoms are more severe with the increasing number of X-chromosomes.

(*d*) Presence of an extra Y-chromosome in males (XYY). Such individuals have unusual height, mental retardation and criminal bent of mind Their genitalia are affected by developmental abnormalities.

The nature of the syndrome can be easily determined by sex chromatin or Y-body analysis from the buccal mucosa and epithelial cells from hair roots. One of the two X-chromosomes of a normal female becomes heterochromatic and appears as a chromatin body (stainable by orcein) in the interphase nucleus (Fig. 22.6). This body

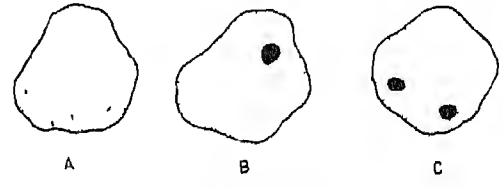


Fig. 22.6 Barr bodies in diploid cells of human beings. *A*. A cell from a normal male has no Barr body. *B*. A cell from a normal female has one Barr body. *C*. A cell from an individual with three X-chromosomes has two Barr bodies.

is known as the Barr body. The interphase nuclei of males do not have this body because each cell has only one X-chromosome. In cells with a higher number of X-chromosomes, the number of Barr bodies increases correspondingly. Thus, cells with three X-chromosomes have two Barr bodies, cells with four X-chromosomes have three Barr bodies, and so on. Similarly, the number of Y-chromosomes also can be determined in the interphase cells because the Y-chromosome has a brightly fluorescent band on its long arm which appears as a fluorescent spot under ultra-violet light in the interphase nuclei that are stained with quinacrine dyes. Thus, the number of Y-

and X- chromosomes associated with various abnormalities can be determined by counting the number of Y spots and Barr bodies in the interphase nuclei (Table 22 1).

Abnormalities Due to Multiple Sets of Genomes

Normal human beings are diploid (2n=46) with two sets of genomes. Sometimes, more than two sets are present. Individuals with 3 (3n=69), 4(4n=92) and 8 (8n=184) sets of chromosomes show many phenotypic abnormalities.

common examples of sex-linked disorders. The special feature of sex-linked inheritance can be understood by studying the transmission of hemophilia.

The X-chromosome of males and females has a gene which controls the production of a vital factor required for the quick clotting of blood during bleeding. Males have only one X-chromosome in each cell and, therefore, only one gene of this kind, which produces the coagulant. Sometimes, this gene mutates and the production of this vital

TABLE 22.1

Number of Barr Bodies and Y Spots, and Phenotypes of Human Beings with Different Constitutions of Sex Chromosomes

Sex chromosome	*Number of Barr bodies*	*Number of Y spots*	*Phenotypes*
FEMALE			
XO	0	0	Turner's syndrome
XX	1	0	Normal
XXX	2	0	Super female with mental abnormalities
XXXX	3	0	Super female with mental abnormalities
XXXXX	4	0	Super female with mental abnormalities
MALE			
XY	0	1	Normal
XYY	0	2	Normal
XXY	1	1	Klinefelter's syndrome
XXYY	1	2	Klinefelter's syndrome
XXXY	2	1	Extreme Klinefelter's syndrome
XXXXY	3	1	Extreme Klinefelter's syndrome

Abnormalities Due to Gene Mutations

Various diseases in human beings are caused because of mutations leading to loss of functions or abnormal functions of some of the vital genes. Mutations may occur in genes of sex chromosomes or of autosome. In the former case, the affected trait shows sex-linked inheritance or is expressed in a particular sex only. Hemophilia and red-green colour blindness are some of the

factor is hampered. As females have XX-chromosomes, the defect in the gene of one of these chromosomes is not expressed in the presence of the wild type allele on the other chromosome and the individuals remain unaffected. But such females are carriers of the disease (XX^h). A carrier female married to a normal male (XY) can produce the following type of progeny: XX, XY, X^hX and X^hY (Fig. 22.7). X and XY progeny

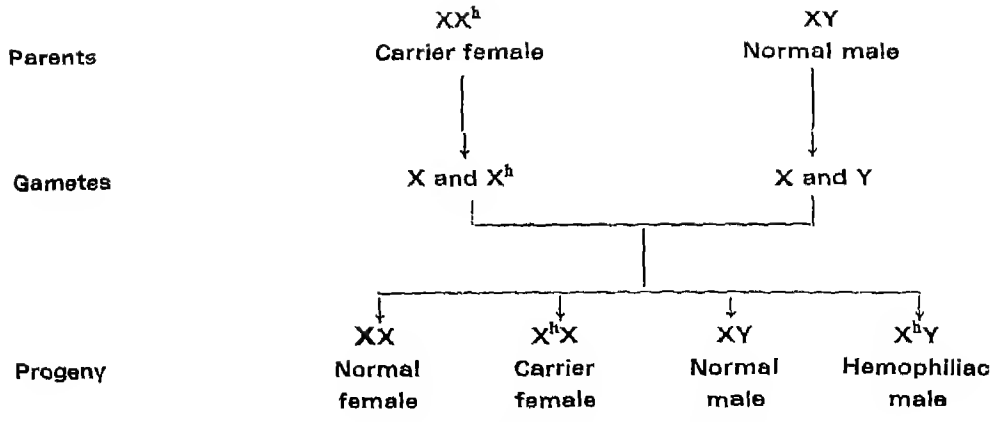


Fig. 22.7 A pedigree to show sex-linked inheritance of hemophilia in human beings.

will be normal female and male, respectively. X^hX will be a carrier female and X^hY will be a hemophiliac male, because the Y-chromosome of the male does not have the corresponding gene to counteract the defective one on the X-chromosome. Many X-chromosome genes do not have their alleles on the Y-chromosome. Females become hemophiliac only when both the X-chromosomes carry the gene for hemophilia (X^hX^h). This will happen if a carrier (X^hX) or hemophiliac (X^hX^h) female is married to a hemophiliac male (X^hY). In the former case, only 50 per cent of the female progeny will be hemophiliac. But in the latter case, all the female progeny will be hemophiliac.

Hemophilia is commonly known as the "bleeder's disease". It is now known that there are two kinds of hemophilia: (1) Hemophilia A due to the lack of antihemophilic globulin, and (2) Hemophilia B due to the lack of plasmathromboplastin. Up to this date, no permanent cure is known for hemophilia. Hemophilia is a sex-linked character because it is inherited through the sex chromosome. It passes from the carrier mother to the son. Hemophilia arises as a result of a recessive mutation in a wild type gene, and once arisen, it is transmitted through innumerable generations until it is lost as a result of back mutation or due to the death of the carrier or the patient.

Disorders Due to the Incompatibility of Genes

So far, we have discussed about heritable disorders which are caused by changes in the number of chromosomes or due to addition, loss or change in the arrangement of chromosome segments or set of genes or due to mutations in genes. Disorders can also be caused as a result of the union of normal gametes from normal parents. In other words, normal parents can have abnormal or lethal children. This happens because of the incompatibility of the genes of the two parents.

No two human beings are exactly alike because different persons contain different sets of genes. The genes direct the synthesis of chemical substances. Therefore, the chemical substances of two individuals are also not exactly alike. Disasters come when two individuals, carrying different incompatible chemical substances, are married. Of the various chemical substances, two are well known for bringing about such defects: one

is the Rh factor and the other is the ABO blood group. Both, the Rh factor and the blood group, are genetically controlled and specify the characteristics of the blood. Simple chemical tests of the blood are available to characterize it with a view to finding out its compatibility with another sample of the blood.

Rh Factor: It was discovered in 1940 that the surface of the red blood cells in some individuals contains a protein which is also present in the blood of Rhesus monkey (that is why it is called the Rh factor). About 85 per cent of the American population possesses this factor or is Rh-positive (Rh^+) and about 15 per cent lacks it or is Rh-negative (Rh^-). Genetical studies have shown that the formation of the Rh protein is controlled by a dominant gene, which has been designated as R. Therefore, RR (homozygous dominant) and R_r (heterozygous) individuals are Rh-positive and rr (homozygous recessive) individuals are Rh-negative. Rh-positive as well as Rh-negative individuals are phenotypically normal. The trouble arises when the blood of a Rh-negative individual comes in contact with the blood of a Rh-positive individual, either due to blood transfusion or during pregnancy.

If the blood of a Rh-negative person is not in earlier contact with the blood of a Rh-positive person, the first transfusion of the Rh-positive blood will not do any harm, since the Rh-negative person grows anti-Rh factors in his own body. But if the second transfusion of the Rh-positive blood is given, immediately the already grown anti-Rh factors will attack the donor's blood. The situation may be worse if a Rh-negative pregnant woman has a Rh-positive baby in her womb (Fig. 22.8). If she did not have any previous contact with the Rh-positive blood either through blood-transfusion or through previous pregnancy, her first child

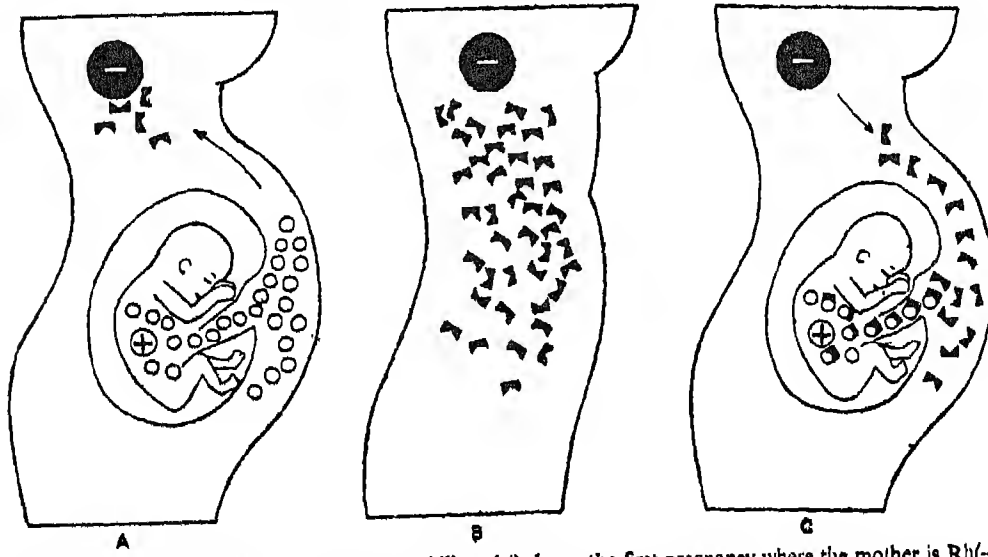


Fig. 22.8 The mechanism of Rh incompatibility. (*A*) shows the first pregnancy where the mother is Rh(—) and foetus Rh(+). Protein (empty circles) of the foetus stimulates the production of anti-factors (black blocks) in the mother. (*B*) shows the retention of anti-factors in the mother's body. (*C*) shows the Rh(+) foetus in the womb of the same mother during the second pregnancy. The anti-factors from the mother's body will destroy the infant's red blood cells.

will be safe. The Rh-positive blood from the foetus will only stimulate the production of anti-Rh factors in the mother's blood. Enough anti-Rh factors will not be produced during the period of pregnancy and the first child will escape the danger. At the time of the second pregnancy, if the child is again Rh-positive, the anti-Rh factors from the mother's blood will attack and destroy the red blood cells of the embryo. Consequently, the child will be anaemic and will show various kinds of developmental abnormalities. For the sake of simplicity, only two variations of the Rh-factor have been described here. In fact, there are many more. Each variant is controlled by a different allele and is heritable.

ABO Blood Group: In addition to the Rh factor, the surface of the red blood cells of man may contain other types of proteins called A and B. According to the presence or absence of these proteins, human beings may be of the following blood groups:

(1) Group A — having only protein A and the anti-factor for B.
(2) Group B — having only protein B and the anti-factor for A.
(3) Group AB — having both A and B proteins and the anti-factor for none.
(4) Group O — having neither A nor B protein but having the anti-factor for both.

These blood groups are controlled by various forms of the gene L, which can be of three kinds: L^A, L^B or L^O. Each individual has any two of these three alleles. Thus, the genotypes of the above-mentioned four blood groups will be as follow:

Blood Group A — L^AL^A or L^AL^O
Blood Group B — L^BL^B or L^BL^O
Blood Group AB — L^AL^B
Blood Group O — L^OL^O

Like the individuals with different Rh-factors, the individuals of different blood groups are perfectly normal. But the incompatibility of some groups is expressed either during blood-transfusion or during pregnancy. Table 22.2 shows the various combinations of blood groups that are tolerated.

It is clear from the table that Blood Group AB can receive the blood from any group but can donate only to its own group. The individuals of this group are, therefore, known as universal recipients. Similarly, the individuals of Blood Group O are universal donors. Universal recipients and donors are so, provided the Rh-factors are compatible. Transfusions of the blood of incompatible groups result in serious reactions. The incompatibility of blood groups results in more serious trouble during pregnancy. For example, the foetus of Group B in the body of the mother having Group A is attacked by the anti-factor B of the mother. This leads to abnormalities like anaemia, jaundice, etc.

The knowledge of the mode of inheritance and genetic control of various human disorders helps in preventing and eliminating them. Some of these aspects have been discussed in the next chapter.

TABLE 22.2
Characteristics of Various Blood Groups and Tolerable Combinations

Blood group	*Anti-factor present*	*May donate blood to*	*May receive blood from*
A	Anti-B	A, AB	A, O
B	Anti-A	B, AB	B, O
AB	None	AB	A, B, AB, O
O	Anti-A and Anti-B	A, B, AB, O	O

EXERCISES

1. Enumerate the modern approaches to human genetics
2. What is the difference between the karyotype of a man and that of a woman? How does this difference determine the sex of the child?
3. What do you mean by the banding pattern of chromosomes? How is it helpful in human genetics?
4. Name a few of the autosomal and sex-chromosomal abnormalities in man and indicate their chromosomal bases and symptoms.
5. Write short notes on the following:
   (*a*) Barr body, (*b*) Turner's syndrome, (*c*) Down's syndrome,
   (*d*) Klinefelter's syndrome, (*e*) Universal donors.
6. With the help of a suitable pedigree chart, indicate the possible genotypes and phenotypes of the progeny of a marriage between a colour-blind man and a normal women.
7. A recessive mutation is more easily expressed in males than in females. Why?
8. What is the Rh-factor? When is an individual Rh-positive?
9. What happens when a Rh-negative mother bears a Rh-positive child.
10. What will be the blood group of the following genotypes?
    (*a*) L^AL^B, (*b*) L^BL^B, (*c*) L^OL^O, (*d*) L^AL^O, and (*e*) L^BL^O
11. What will be the blood groups of the children of the following matings?
    (*a*) $L^AL^B \times L^BL^B$, (*b*) $L^AL^O \times L^AL^B$, (*c*) $L^AL^B \times L^AL^B$, and (*d*) $L^OL^O \times L^AL^B$.

CHAPTER 23

Genetics and Society

THE KNOWLEDGE gained from the study of the science of heredity and variation has been used for the improvement of plants and animals for the benefit of man. It has been used for tailoring the genotypes and phenotypes of individuals and populations to suit human requirements. It has also been used to combat various human diseases and to improve the quality of life. Some of these achievements and future prospects are discussed in this chapter.

Improvement of Plants

Man is entirely dependent on plants because only the latter have the capacity to use solar energy for the synthesis of various organic compounds which are used by man and animals as sources of energy. The very survival of man is dependent on the quantity and quality of plant resources. It is, therefore, not surprising that since time immemorial man has been trying to improve the economically important plants. The scientific improvement of plants is known as *plant breeding*. As civilization progressed, man learnt to cultivate useful plants and he selected the seeds from the stouter and healthier plants for sowing in the next year. Thus, selection emerged as the earliest method of plant improvement. Even at present this method is practised on a large scale, but, of course, with a wider scientific background. Over the years, farmers of different regions have selected varieties of different crops that are suitable for local conditions. The greatest limitation of this method is that selection can be made only from the range of genetic variability that is present in a population. At the same time, it is difficult to ascertain at the time of selection whether the improved phenotype is controlled by the environment or the genotype. This is ascertained only during subsequent generations.

The other method of plant improvement is introduction. It is well established that each crop originated at only one, or a few locations in the world. Professor N. I. Vavilov of the USSR proposed that there are eight major centres of origin of cultivated plants: the Chinese Centre, the Hindustan Centre, the Central Asiatic Centre, the Near Eastern Centre, the Mediterranean Centre, the Abyssinian Centre, the South Mexican and Central American Centre, and the South American Centre. The bread wheat originated in the Central Asiatic Centre, rice in the

Hindustan Centre and potato in the South American Centre. Most of the crops are now grown all over the world under varied conditions. This is because they were introduced in new areas by visitors and traders. For example, potato, maize and tobacco were introduced in Asia by traders from America. Some recent introductions in India include the dwarf rice variety (Taichung Native 1) from Formosa (now Taiwan), the International Rice-8 (IR-8) from the Philippines, and the triple gene dwarf wheat varieties (Sonora, Lerma Rajo, etc.) from Mexico. Norin, the gene for dwarfness, arose in Japan from where it was introduced into the USA, then into Mexico and later into India.

With the knowledge of sexuality and fertilization in plants, the practice of hybridization started. The first successful plant hybrid was obtained by Thomas Fairchild in 1717, by crossing together sweet william and carnation. Rediscovery of mendelism during the early years of this century laid the foundations of scientific and aimed hybridizations for the improvement of crops. At present, efforts are being made at national as well as international level to step up the quality and quantity of food in the face of increasing world population and dwindling resources. Judicious combination of introduction, selection and hybridization has resulted in the development of improved varieties of almost all economically important plants. It will not be an exaggeration to say that all the cultivated land of the world is at present under scientifically improved varieties of crops.

As a result of hybridization, the desirable characters of two or more species or varieties are combined together, or are transferred from one to the other. It involves emasculation or removal of anthers of female parents before dehiscence, its protection from unwanted fertilization, and collection and transfer of the pollen from the male parent to the stigma of the emasculated flower. Unisexual flowers, like those of maize, do not need to be emasculated. Similarly, emasculation is not needed if the female parent is self-sterile or self-incompatible. Details of the various manoeuvres of hybridization depend on the structure and physiology of the floral parts. In maize, since the hybrids show increased vigour, they are grown as crops. In other plants, subsequent generations are raised from the hybrids and suitable recombinants are selected, tested and multiplied before being distributed to the farmers. Hybridization accompanied with induced mutation, polyploidy and chromosomal aberration generates more variation in a population

Fig. 23.1 Triticale — the man-made cereal.

and this provides a wider choice for selection. As a result of hybridization between wheat and rye, man has been able to synthesize a new cereal — *Triticale* (Fig 23.1)

During recent years, many new techniques have been developed to transfer desirable characters from one organism to the other or to tailor the genotype of a given species. These are discussed later in this chapter under 'genetic engineering'.

Improvement of Animals

India has a large number of livestock. Still milk, meat, egg and other animal products are in short supply because we do not have improved strains or breeds of animals in sufficient numbers. Controlled breeding between two desired breeds followed by judicious selection results in improved breeds. As the number of superior breeds available in our country is limited, artificial insemination is resorted to.

In order to improve the milk yield per cow and in order to have hard-working buffaloes, a large number of foreign breeds have been introduced into India. Some of these are: Jersey (England), Ayrshire (Scotland), Brown Swiss (Switzerland), Holstein, Freisian (Holland), etc. Improved hybrid varieties like Jersey-Sindhi, Ayrshire-Sahiwal, Brown Swiss-Sahiwal, etc., have been developed after controlled breeding. The hybrid cows give much more milk than the pure breeds and the hybrid oxen are more hardy, laborious, energetic and agile. Of late, the Ayrshire-Sahiwal breed has become unpopular because it loses the hybrid vigour in successive generations.

Hybridization by artificial insemination was first introduced in India in 1944 at the Indian Veterinary Research Institute, Izatnagar. Normally, a bull can produce only 50 to 60 calves per year by natural mating By artificial insemination, it is possible to produce about 1000 calves in a year from one bull. The general procedure of artificial insemination includes collection of semen, its examination, preservation, transport and injection into the female and study of the results of insemination. Artificial insemination has many advantages It is very economical and makes possible a wider use of the superior bulls. The semen from bulls located at distant places can be used. The spread of diseases can also be controlled.

Other animals like chicken, ducks, pigs, etc , have also been improved by introduction and controlled breeding Aseel, Chittagong and Ghagus are some of the *Desi* breeds of fowl, whereas White Leghorn, Rhode Island Red, Black Minorca, etc., are the introduced ones. Quite a few hybrids between the *Desi* and introduced breeds are available in the country. Boars of foreign breeds like those of Large White Yorkshire, Middle White Yorkshire and Berkshire have been used to improve Indian pigs which are slow growers and the pork of which is of low quality. Pig-breeding and management is a very lucrative business in western countries because these animals are most prolific breeders and quick growers. They are the most efficient converters of feed into meat.

Conservation of Gene Pool

Genes exist in association with other genes in an individual. Different individuals have different sets of genes. Many individuals comprise a population. The sum total of genes in a population is called the gene pool. Hereditary changes in individuals are reflected in changes in the gene pool. Individuals differ a great deal from each other in their reproductive ability and die after a limited period of growth, differentiation and reproduction. But the population and the gene pool are maintained within certain limits during a short span of time. The population structure

as well as its genetic make-up changes over a number of generations and this leads to evolution of the species. These changes involve types as well as frequencies of genes in a population. Gradually, some genes disappear and new ones appear. Many genes of wild populations have been found to be useful and have been transferred to cultivated plants and domesticated animals. In recent times, therefore, there has been an increasing concern about the preservation of natural gene pools. Nature is the best conservatory, but due to man's encroachment it is dwindling very fast. Efforts are being made by national as well as international organizations to collect and preserve useful germplasms. The Central Rice Research Institute at Cuttack maintains a collection of over 8,000 strains of rice and the Sugarcane Breeding Institute of Coimbatore has a large collection of sugarcane varieties. Huge collections of wheat, maize, rice, soyabean, etc., are being maintained by the plant Introduction Division of the United States Department of Agriculture. Similar collections of important crop varieties are being maintained in the USSR and other countries. Wild life sanctuaries and national parks help in preserving gene pools.

Genetic Counselling

Man has never been as deeply concerned about his future as he is now. He is making all-round efforts to improve his health, to have better and healthy progeny and to have enough of good quality food. He is being helped by geneticists in achieving his goal. Laboratory scientists do fundamental researches and explore the possibilities of applications of new findings. Genetic counsellors help in educating the public about the use and misuse of various inventions. They predict the characteristics of the future generation progeny and, thus, help in planning the parenthood

The counsellor can determine the probability of having a child with a certain hereditary defect. The karyotype analysis of parents can reveal if they have any chromosomal abnormality which can be transmitted to subsequent generations Biochemical tests can reveal if there is any incompatibility of the urine. blood, etc , of the husband and the wife Such tests, carried out before marriage, can prevent many miseries The sex of very young unborn embryos can be determined by examining a sample of cells from the fluid surrounding them in the womb. This can help in predicting the occurrence of sex-linked disorders in the progeny. Suspected errors of metabolism and hereditary disorders can be prevented by voluntary abortions Many affluent countries have very well-staffed genetic counselling centres.

The advice of geneticists is also taken before releasing or withdrawing varieties of economically important plants.

The role of genetic counsellors will become more important with the perfection of the techniques of genetic engineering.

Genetic Engineering

Genetic engineering aims at adding, removing or repairing a part of the genetic material, thereby changing the phenotype according to will Breeding is the oldest and the most well-utilized technique of genetic engineering. During recent years, quite a few new methods and techniques have been developed by which the genetic material can be manipulated They promise a bright future.

The first step in genetic engineering is the isolation of the desired genetic material. The techniques of extraction and purification of DNA from various sources are so well established that it has become a classroom experiment. The excitement over engineering

the genetic material has enhanced a great deal since Har Govind Khorana (who shared the 1968 Nobel Prize in Medicine with M. Nirenberg and R. Holley) perfected the technique of test-tube synthesis of a known sequence of nucleotides or a gene. Once synthesized, the gene or a genetic segment can be multiplied with the help of replicating enzymes and the mixture of bases. Another breakthrough in this direction has been the isolation and purification of specific segments of DNA from a living organism. This has been achieved by Beckwith and his colleagues of the USA. They have been able to isolate and purify the *lac* genes of the bacterium *Escherichia coli* (Fig. (23.2). This set of genes

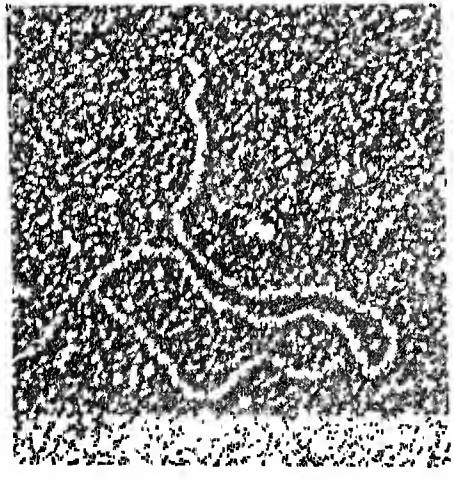


Fig. 23.2 The purified *lac* gene as seen under an electron microscope.

is concerned with lactose utilization for the bacterium.

The next step in genetic engineering is the transfer of the genetic segment from one organism to the other or from the test-tube to the cell. The well-established method to achieve this is by transformation. Transformation is the process by which a cell or tissue or organism takes up the segments of the naked DNA from its surroundings, incorporates it in its hereditary material and ultimately expresses the character specified by the incoming DNA. Transformation has been achieved in a number of plants and animals. The tough cell wall of plants was at one time supposed to be a barrier to the uptake of DNA, but this difficulty has been overcome by digesting the cell wall either completely or only partially with the help of suitable enzymes. With the synthesis and purification of genes, the process of transformation has become more controlled and precise.

Another established method of genetic transfer is by transduction. Transduction is a process, originally discovered in bacteria, wherein a virus (bacteriophage) infects a bacterium, carries a part of the bacterial genome while coming out of it, infects another host, thereby transferring a genetic segment from one individual to the other. Transduction has been found to occur in higher organisms, too. Thus, SV 40, a virus that attacks human beings, can transfer a genetic segment from one host to the other. Transduction has been used to transfer the *lac* genes of *E. coli* to haploid callus of *Arabidopsis* and tomato. The carrier of genetic material in these cases has been the *lambda* bacteriophage.

More recently, plasmids have been utilized for manipulating the genetic material. Plasmids are rings of DNA that occur frequently in bacteria, over and above the main genome. They carry genes for sexuality, antibiotic resistance, etc., but not any vital gene, so that a cell can survive even without them. Plasmids replicate independently of the main genome and, being small, can easily come out of or get into a cell. Besides the plasmids, two newly discovered groups of enzymes — the restriction endonucleases (R.E.) and

ligases — are also used for genetic engineering. The restriction endonuclease is used to splice the plasmid as well as the foreign DNA molecule at specific points in such a manner so as to have sticky ends in both the molecules. The free sticky ends of the plasmid DNA and the foreign DNA serve as convenient points for their complementary pairing The gaps are then sealed by a ligase, thus making a circular DNA piece which contains the plasmid genes as well as a piece of foreign DNA (Fig. 24 3) Such a recombinant DNA can be introduced into a bacterial cell as a plasmid, where it can replicate and express itself. Using this technique, the ribosomal genes of *Xenopus* (a toad) have been incorporated in the cells of the bacterium *E. coli* It has also been possible to transfer the globin gene (the gene which codes for the protein part of haemoglobin) of a rabbit and the insulin gene of a rat to the same bacterium Evidences have been gathered to prove that the new DNA is transcribed in the bacterial cell. News about the final synthesis of relevant proteins is eagerly awaited.

There have been many achievements of genetic engineering and there are prospects of achieving things which are at the moment in the realm of science fiction and human fantasy. Some of these can be enumerated as follow:

1. Genetic engineering puts us at the threshold of a new form of medicine, gene therapy, to treat crippling hereditary diseases like homophilia, phenylketonuria, etc.

2. The introduction of genes coding for vitamins, antibiotics or hormones from higher animals to bacteria opens up the possibility of the creation of living factories churning out chemicals that are otherwise difficult or impossible to get or to synthesize

3. This technology also opens up the possibility of the transfer of nitrogen-fixing genes from bacteria or blue-green algae to our major food crops, thereby enabling them to fix atmospheric nitrogen. This will greatly increase the world food supply and will also make us independent of expensive synthetic fertilizers.

4. It may well be possible to produce plants and animals of totally a new design and to tailor their characteristics according to will

5 Besides the above-mentioned practical benefits, genetic engineering permits a thorough study of the nature and function of the hereditary material It provides a way to find the location of specific genes within the chromosome and gives a deeper insight into when and where enzymes are made.

But the potential benefits of this technology must be weighed against its hazards There is a positive danger that the manipulation of genes might, by accident, result in the origin of new kinds of diseases and organisms containing fatal genetic elements. These may escape and contaminate the entire earth and may not reveal its presence until its deadly work is done Many drugs, such as antibiotics, may become ineffective if the bacteria acquire resistance due to uncontrolled recombinant DNAs. There is also the fearsome possibility that the politicians may misuse this technique to serve their own ends to create Frankensteinlike monsters or Hitler-type ruthless dictators.

Protoplast Fusion

In conventional breeding experiments, only related species can be made to mate. Crossing between unrelated species and genera is difficult and very often impossible. This difficulty has been overcome by the discovery of the technique of protoplast fusion. It was in 1965 that H. Harris and J. F. Watkins of Oxford reported for the first time that cells from different animal species (mouse and man) can be made to fuse to form hybrid

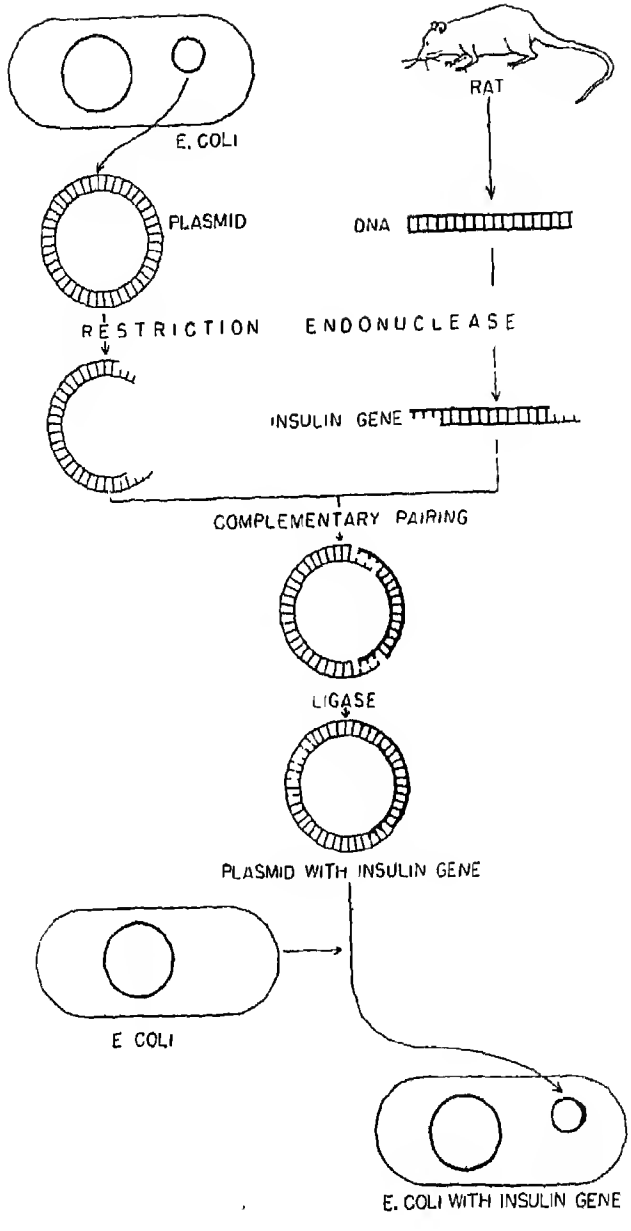


Fig. 23.3 Steps involved in the transfer of the insulin gene from a rat to *E. coli*. Rat insulin gene was isolated by using a restriction endonuclease. Plasmid was isolated from *E. coli* and treated with the same endonuclease, thereby exposing complementary bases The isolated insulin gene was then joined to the plasmid with the help of ligase The plasmid with the insulin gene was introduced into an *E coli* cell

cells. The first reaction to this report was that cells from different animal species could be fused together to form viable hybrids (Fig. 23.4). Since then, there have been many reports of fusion of cells from different animals but so far no hybrid animal has been produced by this technique, because animal cells fail to differentiate under culture conditions. On the other hand, at least two interspecific plant hybrids have been produced by this technique. One of them is a hybrid between two different species of tobacco (Fig. 23.5) (Carlson *et al.*, 1972) and the other one is a hybrid between two different species of *Petunia* (Power *et al.*, 1976). More recently, there have been reports about the fusion of plant protoplasts and animal cells. Cocking and his colleagues (1975) have been successful in fusing yeast protoplasts with hen erythrocytes, and Dudits *et al.* (1976) have reported on the fusion of human cells with carrot protoplasts. It will be interesting if the plant components of these hybrids bring about differentiation.

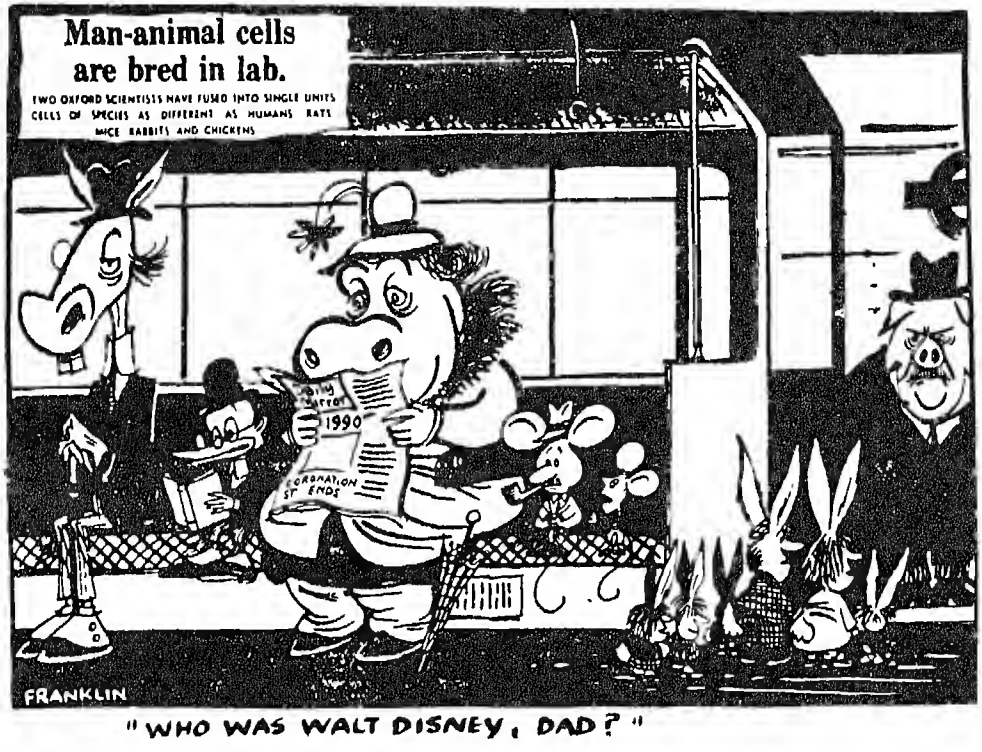


Fig. 23.4 A cartoon published in *Daily Mirror* in response to the first announcement that cells from different animals can be made to fuse to form hybrid cells.

Protoplast fusion opens up the possibility of overcoming the sexual barriers and of mixing and reassorting the genetic elements of hitherto sexually isolated organisms. It offers many exciting vistas for the genetic manipulation of plants. Hybridization through protoplast fusion is known as parasexual hybridization because it does not involve sexual fusion.

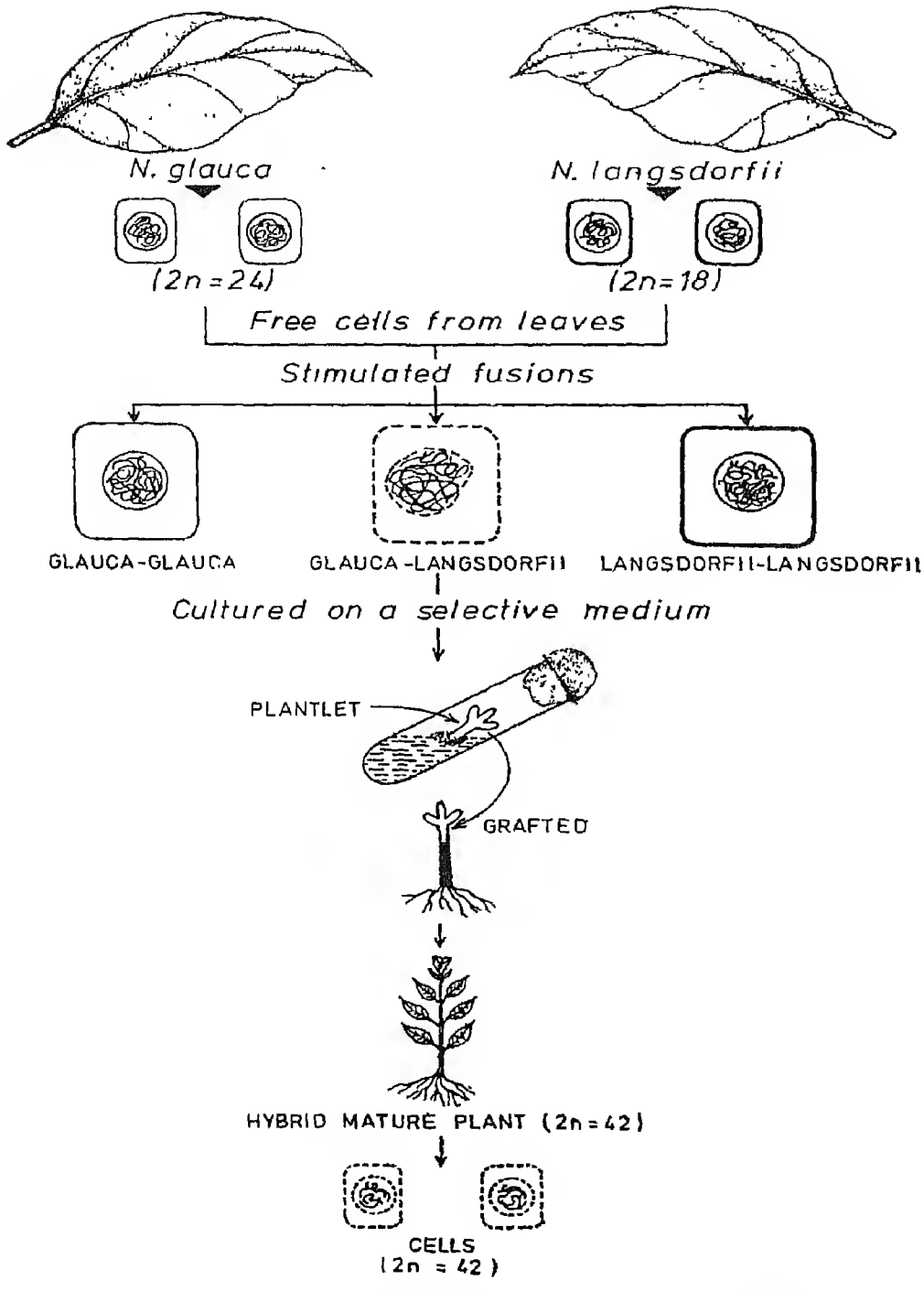


Fig. 23.5 Scheme of hybridization by protoplast fusion in tobacco.

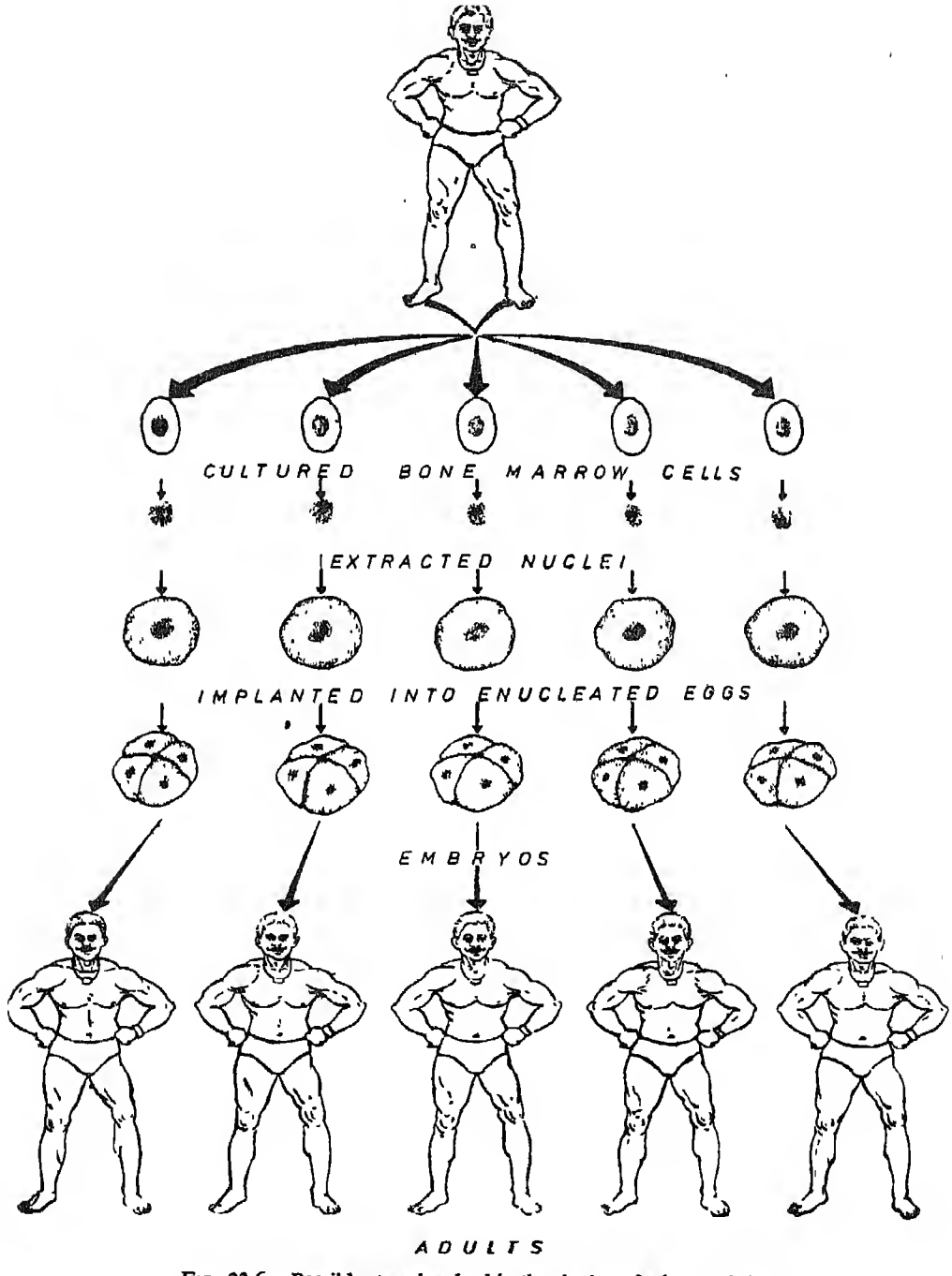


Fig. 23.6 **Possible steps involved in the cloning of a human being.**

Cloning

Genetic engineering can correct hereditary defects or can improve the human race, but it cannot prevent death. With the death of an individual, a particular genotype (with a particular set of genes and characters) is lost. One way of preserving the genotype is to clone the individual. A clone is a population of cells or individuals which are genetically identical. Clones of plants are easy to get by vegetative propagation or by the tissue culture methods. An extension of the same technique in human beings can result in one or many replicas of an individual (Fig. 23.6.. The technique will involve artificial fertilization in a test-tube and induction of growth and differentiation in the zygote thus obtained

Only time will tell whether these rapid strides in genetics and allied sciences ensure a better and happy society. The prospects appear to be bright.

EXERCISES

1. 'Selection and introduction are two important methods of plant improvement'. Comment on this statement
2. Discuss the scope and achievements of plant hybridization.
3. How can genetics help in the improvement of our cattle wealth?
4. Write explanatory notes on
   (*a*) Gene pool, (*b*) Genetic counselling, (*c*) Transformation, (*d*) Transduction, and (*e*) Plasmids
5. Discuss the achievements and prospects of genetic engineering.
6. With the help of a neat diagram, illustrate the steps involved in the transfer of the insulin gene from the rabbit to *E. coli*
7. Why are the scientists as well as the enlightened citizens so much concerned about the experiments on genetic engineering?
8. What do you mean by protoplast fusion? What are the prospects of this kind of work?

APPENDIX

Some Important Contributions to the Study of Cell Biology and Genetics

Name	*Contribution*
H. J. Dutrochet	All plants and animals are composed of cells.
R. Brown	Described the characteristic dancing of cell particles which is now referred to as the Brownian movement.
M. J. Schleiden	Described nucleoli although first noted by Fontana (1781).
M. J. Schleiden and T. Schwann	Formulated the cell theory
R. Virchow	Stated that all cells arise from the pre-existing cells.
W E Waldeyer	Reported the use of the new common haematoxylin to stain tissue cells in which he described chromosomes
G. J. Mendel	Discovered the fundamental principles of genetics.
E. Haeckel	Named plastids.
His	Developed the microtome for cutting sections of tissues for cell study. Preservation of tissues dates from Boyle (1663) who used alcohol as a preservative for specimens.
F. Miescher	Discovered nucleic acid (nuclein).
Fol	Observed a spermatazoan penetration of an ovum
W. Flemming	Introduced the term chromatin and described the splitting of chromosomes. In 1882, he described cell division in animal cells by the term mitosis, and named the aster (1892). He suggested a correlation between nucleic acid and chromatin.
E. Strasburger	Described cell division in plant cells and introduced the modern usage of the terms cytoplasm and nucleoplasm.
Schimper	Named chloroplasts, the special bodies of Sach (1865) and the green granules of Comparatti (1791).
W. Waldeyer	Introduced the term chromosomes.
T. Boveri	Named the centrosome, and in 1892 he published the still current diagrams illustrating spermatogenesis and oogenesis.
C. Benda	Named the mitochondrion.
C. Golgi	Described the Golgi complex as an internal reticular apparatus.
C. E. McClung	Identified the sex chromosomes in the grasshopper.
R. G. Harrison	Developed the technique for growing tissues in culture
A. Kossel	Chemistry of cell nucleus.
T. Svedberg	Ultracentrifuge.
O. H. Warburg	Action of the respiratory enzyme.
T. H. Morgan	Function of chromosomes in transmission of heredity.

Date		*...solution*
1938		Developed ultra-violet photomicrography for the study of nucleic acids.
1944	O. T. Avery, C. H. Mclea and H. McCarty	Showed the significance of DNA in heredity transmission in bacteria.
1946	H. J. Muller	Production of mutations by X-ray irradiations.
1946	J. B. Summer	Crystallized the first enzyme.
1946	J. H. Northrop and W. M. Stancey	Preparation of enzymes and virus proteins in a pure form.
1953	F. Zernike	Phase-contrast microscopy.
1953	H. A. Krebs	Citric acid cycle.
1953	J. D. Watson, F. H. C. Crick and M. H. F. Wilkins	The double helix model for the DNA molecule.
1954	L. C. Pauling	Nature of the chemical bond.
1958	F. Sanger	Structure of insulin.
1958	G. W. Beadle, E. L. Tatum and J. Lederberg	One gene regulates one definite chemical process
1959	S. Ochoa and A. Kornberg	In vitro synthesis of polyribonucleotides, In vitro synthesis of polydeoxyribonucleotides.
1961	M. Calvin	Work on photosynthesis.
1962	J. C. Kendrew and M. F. Perutz	Structure of protein.
1965	F. Jacob, J. Monod and A. Lwoff	Discovery of a class of genes which regulate the activities of other genes.
1968	M. W. Nirenberg, H. G. Khorana and R. H. Holley	Genetic code and base sequence of tRNA.
1969	M. Delbrueck, A. D. Hershey and S. E. Luria	Reproductive pattern of viruses.
1970	B. Ephrusii H. Harris	Cell hybridization. DNA-RNA hybridization technique
1971	E. W. Sutherland	Role of cyclic AMP.
1972	W. H. Stein, S. Moore and C. B. Anfinsen	Structure of ribonuclease.
1972	R. R. Porter and G. M. Edelman	Chemical nature of antibodies.
1974	A. Clande and G. Palade	Ultrastructure of cell.
1974	C. De Duve	Structure and functions of lysosomes.
1975	H. Temin and D. Baltimore	Discovery of reverse transcriptase.
1975	R. Dulbecco	Viruses as causative agents of cancer.
1976	C. Gajdusek and B. S. Blumberg	Cancer research.